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THE ANALYST

THE JOURNAL OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

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Volume 84, No. 997 April 1959

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, April 1st, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Mr. R. C. Chirnside, F.R.I.C.

The following papers were presented and discussed: "A Modified Hydroxamic Acid Method for the Estimation of Total Esterified Fatty Acids in Plasma," by D. M. Morgan and K. J. Kingsbury, M.B., B.S., Ph.D.; "The Determination of Small Amounts of α-Picoline in Pyridine by a Solution Temperature Method," by K. A. Adey, B.Sc., F.R.I.C., and J. D. Cox, B.Sc., Ph.D., A.R.C.S.; "The Separation of Copper, Cobalt and Nickel," by W. D. Duffield; "The Additive Effect of Substituent Groupings in the Chromatographic Behaviour of Phenoxyacetic Acids," by L. S. Bark, B.Sc., F.R.I.C., and R. J. T. Graham, B.Sc.

DEATHS

WE record with regret the deaths of

George Macdonald Bennett
Alan John Durre
Arthur William Williams.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7 p.m. on Friday, February 27th, 1959, in the Lecture Room of the Royal Society of Edinburgh, 22 George Street, Edinburgh 2. The Chair was taken by the Vice-Chairman of the Section, Mr. A. F. Williams, B.Sc., F.R.I.C.

The following paper was presented and discussed: "Recent Advances in the Analytical Chemistry of Plutonium," by F. J. Woodman, B.Sc., A.R.I.C.

WESTERN SECTION

A JOINT Meeting of the Section with the Bristol Section of the Chemical Society was held at 6.30 p.m. on Friday, February 20th, 1959, in the Large Lecture Theatre of the Chemistry Department of the University of Bristol. The Chair was taken by the Vice-Chairman of the Bristol Section, Professor Wilson Baker, M.A., Ph.D., D.Sc., F.R.I.C., F.R.S.

A lecture on "Some Aspects of Phosphorylation" was given by Professor Sir Alexander Todd, M.A., D.Phil., D.Sc., F.R.S.

MIDLANDS SECTION

THE fourth Annual General Meeting of the Section was held at 6.30 p.m. on Tuesday, March 3rd, 1959, in the Mason Theatre, The University, Edmund Street, Birmingham 3. The Chair was taken by the Chairman of the Section, Dr. R. Belcher, F.R.I.C., F.Inst.F. The following appointments were made for the ensuing year:—*Chairman*—Dr. S. H. Jenkins. *Vice-Chairman*—Dr. H. C. Smith. *Hon. Secretary*—Mr. G. W. Cherry, 48 George Frederick Road, Sutton Coldfield, Warwickshire. *Hon. Treasurer*—Mr. F. C. J. Poulton. *Hon. Assistant Secretary*—Mr. R. Adkins. *Members of Committee*—Messrs. B. B. Bach, A. S. Beidas, W. E.

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Drinkwater, W. T. Elwell, W. M. Lewis, Dr. Alison M. G. Macdonald, Messrs. W. I. Stephen and M. Williams. Miss M. E. Tunnicliffe and Mr. H. J. Alcock were re-appointed as Hon. Auditors.

AN Ordinary Meeting of the Section was held at 7 p.m. on Tuesday, March 10th, 1959, in the Gas Showrooms, Nottingham. The Chair was taken by the Chairman of the Section, Dr. S. H. Jenkins, F.R.I.C., F.Inst.S.P.

The following paper was presented and discussed: "The Microbiological Assay of the B Vitamins," by F. W. Norris, Ph.D., D.Sc., A.R.C.S., D.I.C.

MICROCHEMISTRY GROUP

THE Fifteenth Annual General Meeting of the Group was held at 6.45 p.m. on Friday, February 20th, 1959, in the School of Pharmacy, 29/39 Brunswick Square, London, W.C.1. The Chair was taken by the Chairman of the Group, Mr. D. F. Phillips, F.R.I.C. The following Officers and Committee Members were elected for the forthcoming year:—*Chairman*—Mr. F. Holmes. *Vice-Chairman*—Mr. C. Whalley. *Hon. Secretary*—Mr. D. W. Wilson, Department of Chemistry, Sir John Cass College, Jewry Street, Aldgate, London, E.C.3. *Hon. Treasurer*—Mr. G. Ingram. *Members of Committee*—Mr. E. Bishop, Miss M. Corner, Messrs. J. A. Hunter, C. A. Johnson, R. Magee and D. F. Phillips. Dr. L. H. N. Cooper and Mr. H. Childs were re-appointed as Hon. Auditors.

The Annual General Meeting was preceded at 5.15 p.m. by visits to the microchemistry and chemistry laboratories and the microbiological and pharmaceutics laboratories of the School of Pharmacy. It was followed at 7 p.m. by an Ordinary Meeting of the Group, at which Mr. D. F. Phillips, F.R.I.C., presented the Retiring Chairman's Address (see summary below). The Chair at this meeting was taken by the new Chairman of the Group, Mr. F. Holmes, B.Sc., A.R.I.C.

MR. D. F. PHILLIPS first took a brief backward glance at the progress of microchemical analysis in this country over the past quarter of a century, and then focused attention on present and future trends.

On the organic side a fair amount of attention was being paid to the possibilities of devising methods for the elementary analysis of organic compounds on the decimilligram scale. Considerable difficulties had been encountered, and only in a few instances was a simple scaling down of the older procedures possible. In every shift to a smaller scale the fundamental difficulty of the increase in the relative surface and its effect on contamination and on the exchange of gases and vapours through the relatively enlarged liquid – gas interface became more obvious. Novel approaches had had to be made and successful procedures were becoming available for elemental analysis and for the determination of functional groups on samples of from 50 to 200 μg. It was gratifying to know that basic research into such problems was increasing and that the analyst was endeavouring to keep one step ahead of the biochemical and medical research for which such methods were almost certain to be required. In a similar manner the general techniques for the inorganic analysis of microgram quantities had been outlined by Benedetti-Pichler and refined by his co-workers between the years 1937 and 1940, a period during which their eventual application to the isolation and study of the first transuranic elements a few years later could hardly have been forseen.

Another trend that organic micro-analysts should watch was the use of radiation from artificial isotopes as a cheap, rapid and simple means for the routine determination of elements and groups in organic compounds. The determination of sulphur in petroleum by measurement of its absorption of soft X-rays from iron-55 had been in use in a refinery in the United States of America for more than 5 years.

In the field of inorganic micro-analysis there was, of course, the marked trend towards the use of purely physical techniques, and this was bound to increase. Nevertheless development of essentially chemical methods was still being actively undertaken, with approximately 60 per cent. of the interest focused on the use of organic reagents. The progress of microchemical analysis, and, indeed, analytical chemistry as a whole, had to depend to a very great extent on the discovery of new reagents, new reactions and new methods of separation.

Chromatography, ion exchange and solvent extraction appeared to predominate as techniques for microchemical separations. Interest in gravimetric methods had declined markedly.

The search for more specific and more sensitive reagents was bound to continue. Although, under especially favourable conditions, as little as 10^{-15} g of an element had been detected, there was still a long way to go before the theoretical ultimate limit of detection at somewhere around 10^{-20} g was attained.

The determination of trace constituents at the microgram level did not necessarily always involve the use of true microchemical techniques. A problem that sometimes arose, however, was the determination of traces in milligram amounts of sample. This necessitated the detection and determination of a constituent at the nanogram level. Perhaps in the ominous year of 1984, a quarter of a century forward, we should be called upon regularly to undertake the determination of picogram amounts of an element.

PHYSICAL METHODS GROUP

THE sixty-fifth Ordinary Meeting of the Group was held at 6.30 p.m. on Tuesday, February 24th, 1959, in the meeting room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the Chairman of the Group, Mr. R. A. C. Isbell, A.Inst.P.

The subject of the meeting was "The Analysis of Intact Samples" and the following papers were presented and discussed: "The Use of Beta and Gamma Absorption Methods in the Analysis of Intact Samples," by J. F. Cameron, B.Sc.; "The Use of Radioactive Isotopes in Simple X-ray Fluorescent Analysis," by C. E. Mellish, B.A., D.Phil.; "Analysis by Nuclear Magnetic Resonance Techniques," by D. J. Ferrett, M.A., D.Phil. (see summaries below).

THE USE OF BETA AND GAMMA ABSORPTION METHODS IN THE ANALYSIS OF INTACT SAMPLES

MR. J. F. CAMERON said that the predominant use of gamma-absorption techniques was by employing the Compton scattering process for determining density. This had the advantage that it provided a non-contact measurement that could be made continuously through the walls of containers such as pipes or tanks, and was applied, for example, to petroleum products, ice-cream, acids and slurries of cement, thoria and uranium. The hydrogen to carbon mass ratio of hydrocarbons could be determined.

The photoelectric absorption of low-energy photons was a rapidly varying function of atomic number and hence could be used as an indication of mean atomic number. The scope of the technique was severely restricted, first by the lack of radioactive isotopes emitting low-energy photons, and secondly by the absorption of this radiation in the container walls. The concentrations of uranyl sulphate and uranium "pulp" in aqueous solution and the sulphur and lead concentrations in hydrocarbons could be determined in this way.

Beta-absorption methods were used in gas analysis and smoke detection.

The accuracy of such measurements was usually limited by the statistical variations in the detector output caused by the random changes in the rate of emission of radiation from the radioactive source. The author spoke of the considerations involved in the choice of the detector and source energy, and dealt with the theoretical accuracy, the effect of container-wall thickness and so on.

THE USE OF RADIOACTIVE ISOTOPES IN SIMPLE X-RAY FLUORESCENT ANALYSIS

DR. C. E. MELLISH pointed out that for many applications of X-ray fluorescence spectroscopy the full performance of the conventional spectrometer was not required. In these applications it was possible to substitute a small radioactive source (of the order of 1 millicurie) for the X-ray tube, provided that the crystal spectrometer was also dispensed with and resolution of the fluorescent X-rays produced was performed in a proportion or scintillation counter. Results were given of the application of this simple system to such problems as the measurement of metal-plate thickness and of solution strengths. The suitability of different isotope sources for different applications was discussed.

Analysis by Nuclear Magnetic Resonance Techniques

Dr. D. J. Ferrett said that the phenomenon of nuclear magnetic resonance, in which responses could be obtained from nuclei that have spin, offered a technique for the analysis of intact samples of solids, liquids and gases. Equipment demands were rigorous, *e.g.*, magnets with highly uniform fields and electronic circuits with low noise levels. The information obtained could be both qualitative and quantitative and could be used as an aid to the elucidation of the structure of complex organic molecules.

Obituary

HERBERT JOHN EVANS

Herbert John Evans died suddenly at his home in Carmarthen on December 18th, 1958, at the age of 71. He was a native of Haverfordwest, being the son of the late Mr. John Evans, J.P., of that town, and he was educated at the Haverfordwest Grammar School. He then proceeded to the School of the Pharmaceutical Society in London and later he entered the University College of Wales at Aberystwyth where he met the fellow-student who later became his wife. He obtained the degree of B.Sc. (Wales) in 1910; in the following year he became an Associate of the Institute of Chemistry and in 1914 he was elected a Fellow of the Institute of Chemistry.

After service in France during the first World War with the rank of Captain in the Royal Field Artillery, Evans practised in Liverpool for a number of years, and during that time he was appointed Public Analyst for St. Helens and an Official Analyst under the National Health Insurance Act. In 1943 he took up residence in Carmarthen and quickly built up an extensive private practice there. At the time of his death he was Public Analyst and Official Agricultural Analyst for the Counties of Carmarthen, Pembroke and Cardigan, and Public Analyst for the Counties of Brecknock and Radnor. He was also Official Analyst under the National Health Service Act for the whole of Wales and the South Western Counties of England.

Evans joined our Society in 1920. He was a member of Council of the Institute of Chemistry during the years 1924–27 and he was a founder member and first Chairman of the Western Section of the Society for Analytical Chemistry. He contributed two papers to *The Analyst*, the first in 1936 on "The Estimation of the Original Freezing-point of Sour Milk" and the other in 1956 on "Sucrose Loss from Ice-cream on Storage," both of which occasioned much interest.

Outside his professional life, Freemasonry claimed much of his time. He was Senior Past Master of the Cambrian Lodge, a founder member of Cleddan Lodge, one of the Senior Principals of the Hwllfordd Chapter and a Past Provincial Grand Warden. During his Liverpool days, Evans was a keen yachtsman and during his years at Carmarthen an enthusiastic fisherman.

In a letter to the writer, Colonel W. D. Williams, Vice-Lieutenant of Carmarthenshire, pays the following tribute to his memory—

> "Although his academic achievements had long been known, we did not have the pleasure of meeting Herbert Evans personally until he came to Carmarthen in 1943. His general bonhomie soon endeared him to a wide circle of friends.
>
> As one of his fellow Clubmen recently remarked 'he was a kind friendly person.' A man of upright principles and a devoted Churchman, he will be sadly missed by his professional colleagues and his very numerous friends."

These words express the feelings of all the members of the Western Section who knew "H.J.," as he was affectionately known. He will indeed be missed by those who participate in this Section's activities and particularly at our Annual Summer Meeting, which both he and his wife, Mrs. Eveline May Evans, regularly attended. Their only child, Dr. John Evans, B.A., B.M., B.Ch., died in India in 1945.

The Society was represented at the funeral by his intimate friend Mr. J. G. Sherratt.

Stanley Dixon

The Infra-red Analysis of Solid Substances

A Review*

By G. DUYCKAERTS
(*University of Liège, Belgium*)

Summary of contents

Introduction
Physical factors affecting spectra
Chemical factors affecting spectra
- Adsorption of substance on particles of dispersing medium
- Chemical reaction of substance with medium
- Polymorphism of substance
- Mixed-crystal formation
- Distortion of the crystalline structure
- Formation of complexes between substance and medium

The fundamental principles of the analysis of gases, liquids and solutions by infra-red spectroscopy are well established, and the techniques that have to be used are generally well known. Very early on, infra-red spectroscopists were faced with the problem of finding a method of recording solid-phase spectra. The method based on mulling the sample with Nujol, or with fluorinated or chlorinated hydrocarbons, has been in use for several years and is still standard practice for many purposes, such as quantitative analysis and structural diagnosis.

There are, however, some practical difficulties in the use of solid-phase spectra for quantitative analysis: accurate control of sample thickness, reflectivity and scatter is much more difficult to achieve than with solutions. The internal-standard method[1,2] has been used with success, and a good accuracy has been attained in the analysis of multicomponent mixtures by combining the internal-standard method with the method of compensated extinctions; however, the method runs rapidly into some difficulties owing to Beer's law not being applicable.

The potassium bromide technique, in which the infra-red absorbing solid, finely ground and dispersed in a powdered halide, is pressed into a clear disc, proposed in 1952 by Stimson[3] and Schiedt,[4] was received with much enthusiasm; the absence of adsorption bands in the dispersing medium and the possibility of controlling the sample weight accurately are certainly two important advantages of the disc technique over the mull method. However, I would like to draw attention to the difficulties that are encountered, both in general in quantitative measurements of absorption spectra of solids on the one hand (physical factors), and on the other hand, to the difficulties that arise through the use of alkali halide discs (the chemical and physico-chemical factors).

Physical factors

Every infra-red spectroscopist knows that, beside polymorphism and orientation effects, the two main physical factors that play important roles in infra-red spectra of solids are the dimensions of the individual absorbing particles and the difference in refractive index between the absorbing substance and the dispersion medium (Nujol, potassium bromide and so on).

The influence of the dimensions of the individual absorbing particles is illustrated by Fig. 1, which shows two absorption bands for calcite dispersed in potassium bromide. As the diameter of the calcite particles decreases from 55 to 2·1 μ, there is a progressive reduction of light loss by reflection and scattering and an increase in the intensity of the absorption bands; notice that the last increase in intensity has a greater effect on the intense band at 876 cm^{-1} than on the band at 710 cm^{-1}.

The influence of the refractive index[5] can be seen in Fig. 2, which shows the spectra of poly(vinyl chloride) dispersed in three alkali halides, potassium bromide, chloride and iodide.

* Presented at the meeting of the Midlands Section on Thursday, November 13th, 1958. Reprints of this paper will be available shortly. For details, please see p. 256.

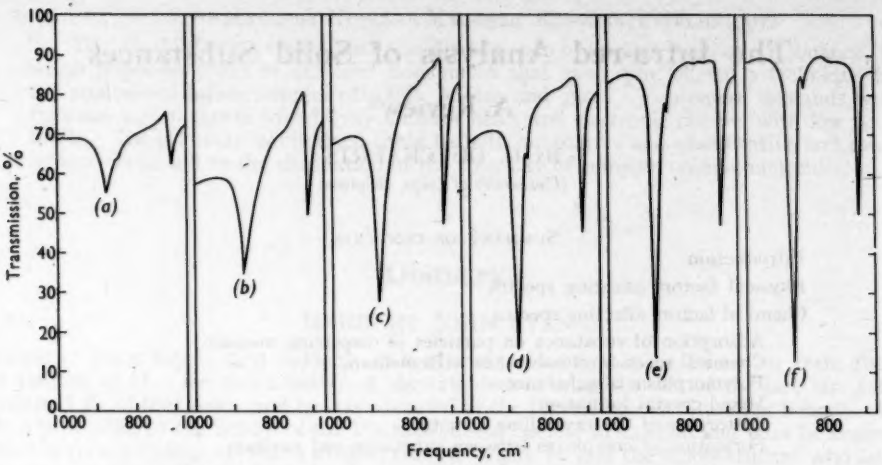

Fig. 1. Absorption bands of calcite in potassium bromide. Mean values for the diameters of the particles: (*a*) 55 μ; (*b*) 40 μ; (*c*) 23 μ; (*d*) 14·7 μ; (*e*) 5 μ; (*f*) 2·1 μ (after Duyckaerts and Lejeune)

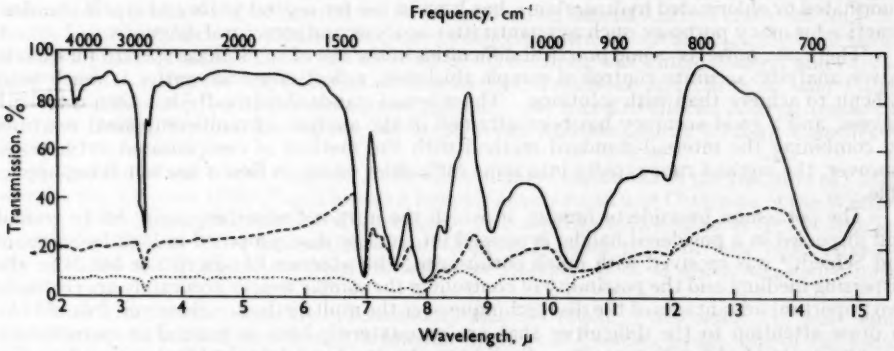

Fig. 2. Infra-red spectra of poly(vinyl chloride) dispersed in potassium chloride (dashes), potassium iodide (dotted line) and potassium bromide (full line) (after Harvey, Stewart and Achhammer[5])

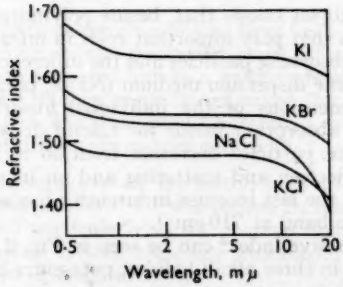

Fig. 3. Refractive indices of the alkali halides

When potassium bromide is the suspending medium, the spectrum is far superior. All sample pellets were of good quality and compensation was applied to eliminate the effects of impurities in the individual salts; spectra of equal quality should have been obtained if it were not necessary to achieve a match of refractive indices between the relatively large sample particles and the suspending medium.

The refractive indices of the alkali halides are shown in Fig. 3; poly(vinyl chloride) has a refractive index, $n_D = 1{\cdot}548$, which is similar to the value of 1·56 for potassium bromide; this indicates that the match is better with potassium bromide than with the iodide or the chloride in the visible region, and the infra-red results shown in Fig. 2 indicate that the same must be true in the infra-red region.

The rapid change in refractive index of a substance in the vicinity of an absorption band often gives rise to the important Christiansen effect, characterised by the asymmetrical shape of the band[6]; for example, the band at 7 μ in potassium chloride (Fig. 2). Fig. 4 shows the C≡N absorption band for cobalt mercury thiocyanate when the substance is suspended in media of various refractive indices (potassium and sodium chlorides, potassium bromide and potassium iodide). The extinctions have been calculated by the base-line method, and one can see that for potassium iodide the shape of the band is nearly symmetrical, whereas for the three other media, there is a pronounced Christiansen effect. As predicted in theory and confirmed experimentally, the importance of the Christiansen effect decreases with the dimensions of the particles, owing to the reduction of loss by scattering. In this example, grinding the substance finely with potassium bromide leads to the production of a spectrum in which the effect has nearly disappeared.

It is also of value to point out that the Christiansen effect gives rise to a shift in the apparent maximum of the band, which in this example moves by about 50 cm^{-1}; this must be taken into account in measuring the frequencies of the absorption bands of solids.

The problem of the influence of particle size on the intensity of the absorption bands has been investigated, during the last few years, by several workers, both from theoretical and experimental points of view.[7,8,9,10,11,12,13,14] The potassium bromide disc technique offered the possibility of more precise quantitative measurements and of changing the refractive index of the suspending medium.. Fig. 5 shows the results obtained for four bands—1431, 876, 710 and 320 cm^{-1}—for calcite; the first three were recorded with a sodium chloride prism, the last with a caesium bromide prism. This figure also shows that for particles having a diameter greater than 15 μ, the apparent extinction is the same for the four bands independently of the value of the true extinction coefficient; this means that in a spectrum of a coarsely ground powder the bands are all of approximately the same intensity. On the other hand, when the dimensions of the particles are reduced to below 10 μ, the apparent extinction starts to increase rapidly, and the limiting value, corresponding to the true extinction of the solid substance, is reached with a particle size that gets smaller as the intensity of the band becomes greater.

It is possible, by a simple calculation, to explain this influence of the particle size on the extinction of an absorption band for the case where there is no difference in refractive index and where all the particles have the same dimensions.

Let us consider a mass, m, of spherical particles of diameter d dispersed randomly in a disc of potassium bromide of cross-sectional area S and length l. The disc may be considered to consist of a number of layers, the number, n, being chosen in such a way that the number of particles in each layer is the same and that a ray of light never hits more than one particle in each layer. With K representing, in each layer, the geometrical fraction of the surface normal to the incident light that is covered by the particles, the fractional transmission for a layer is given by the following expression—

$$\frac{I}{I_0} = (1 - K) + K\theta, \qquad (1)$$

in which θ is the transmission of one particle.

If n represents the number of equal layers, it is easy to calculate the fractional transmission of the disc by assuming that K has the same value in each layer—

$$\frac{I}{I_0} = [(1 - K) + K\theta]^n \qquad (2)$$

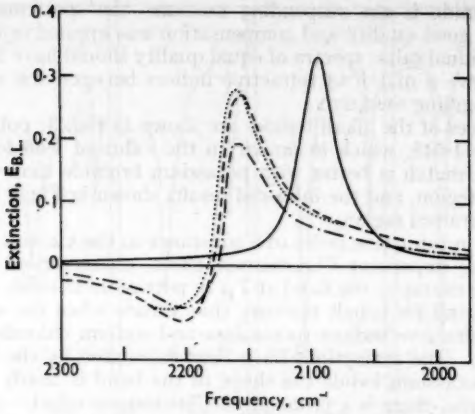

Fig. 4. Absorption band due to C≡N in cobalt mercury thiocyanate, $Co[Hg(CNS)_4]$, dispersed in potassium iodide (full line), potassium bromide (dotted line), sodium chloride (dashes) and potassium chloride (dots and dashes)

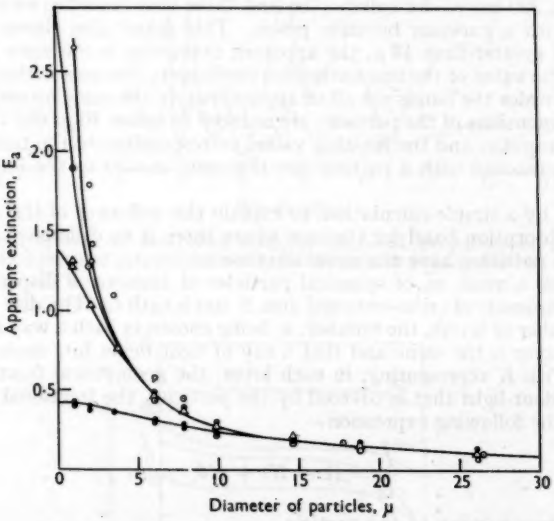

Fig. 5. Extinctions at four wavebands for calcite related to particle size. The lines are the theoretical curves. Experimental values as follows—

○ $\nu = 1431$ cm^{-1} $k = 3{\cdot}5\ \mu^{-1}$
△ $\nu = 876$ cm^{-1} $k = 0{\cdot}99(5)\ \mu^{-1}$
● $\nu = 710$ cm^{-1} $k = 0{\cdot}29(3)\ \mu^{-1}$
⊙ $\nu = 320$ cm^{-1} $k = 2{\cdot}0\ \mu^{-1}$
} $K = 0{\cdot}8$

The following relation exists between n, K, d and the dimensions of the disc—

$$n = \frac{3}{2} \times \frac{m}{\rho SKd}, \quad .. \quad .. \quad .. \quad .. \quad (3)$$

ρ being the density of the substance. From equations (2) and (3), one obtains—

$$E_a = \log\left(\frac{I_0}{I}\right) = -\frac{3}{2} \times \frac{m}{\rho SKd} \times \log\,[(1-K) + K\theta]. \quad .. \quad .. \quad .. \quad (4)$$

On the other hand, the value of θ can be calculated for a sphere as—

$$\theta = \frac{1 - (kd+1)e^{-kd}}{k^2d^2/2} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

and for a cube as—

$$\theta = e^{-kd}, \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

where k is the true absorption coefficient of the solid substance ($i/i_0 = e^{-kd}$).

The limiting value of the extinction may be obtained by expanding the series in equation (4) to get—

$$\lim_{kd \to 0} E_a = \frac{m}{S\rho} \times \frac{k}{2{\cdot}3} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

an equation similar to that for Beer's law ($m/S\rho$ corresponds to cl; $k/2{\cdot}3$ corresponds to ϵ).

Fig. 6 shows the theoretical expression plotted on a semi-logarithmic scale for the example where $K = 0{\cdot}8$ and for two bands with $k = 0{\cdot}6$ and $1{\cdot}0\ \mu^{-1}$; the calculations have been made both for spherical and for cubical particles.

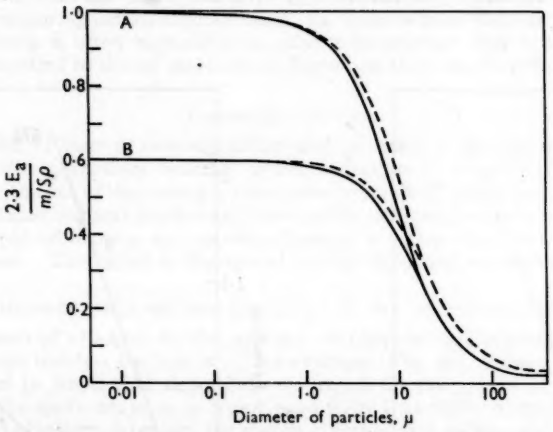

Fig. 6. Theoretical variation of extinction with particle size. Curves A: $K = 0{\cdot}8$, $k = 1{\cdot}0\ \mu^{-1}$; curves B: $K = 0{\cdot}8$, $k = 0{\cdot}6\ \mu^{-1}$. Full line for cubes, dotted line for spheres

We can obtain a still more general equation by introducing into equation (4) the apparent absorption coefficient, $k_a = \frac{2{\cdot}3E_a}{m/\rho S}$—

$$\frac{k_a}{k} = -\frac{3}{2} \times \frac{2{\cdot}3}{Kkd} \times \log[(1-K) + K\theta], \quad .. \quad .. \quad .. \quad (4')$$

an equation containing only two parameters, K and kd (Fig. 7).

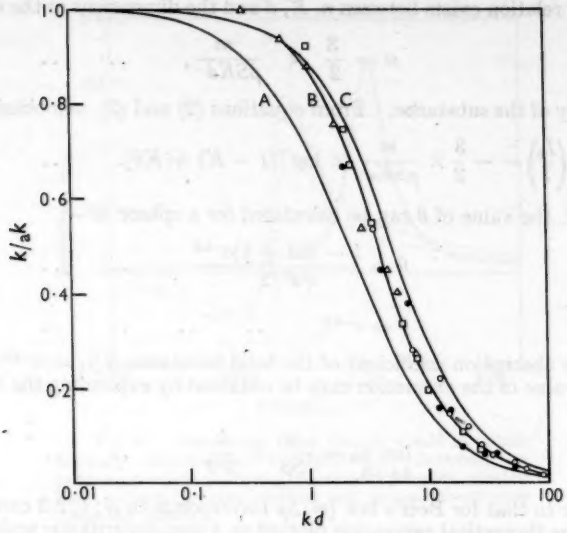

Fig. 7. Comparison of experimental results for calcite with theoretical curves. Curve A, Otvis, Stone and Harp's formula[12] for spheres; curve B, Duyckaerts' formula for spheres; curve C, Duyckaerts' formula for cubes. Experimental points: ○ = 1431 cm^{-1}; □ = 876 cm^{-1}; △ = 710 cm^{-1}; ● = 320 cm^{-1}

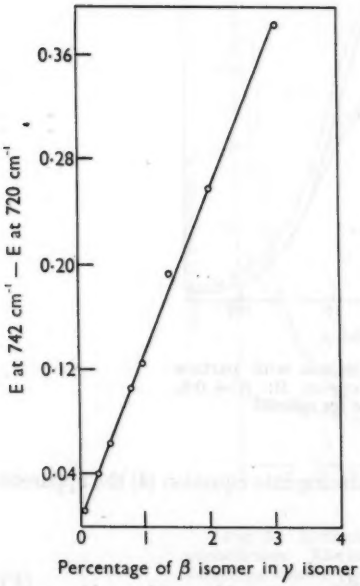

Fig. 8. Calibration graph for the determination of β-hexachloro*cyclo*hexane in γ-hexachloro*cyclo*hexane

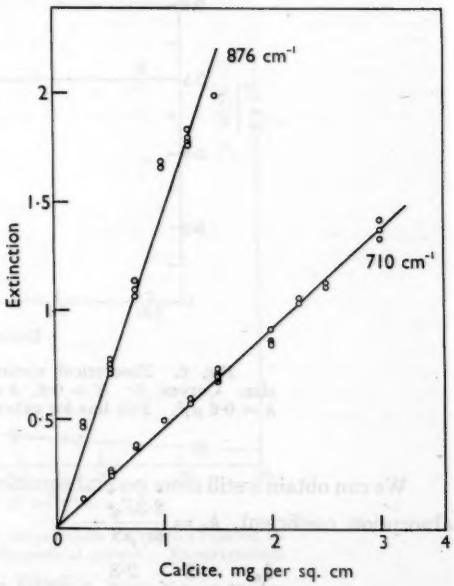

Fig. 9. Calibration graph for calcite

For high values of K (concentrated dispersion), the curve shown in Fig. 7 changes with K, but as soon as $K \leqslant 0{\cdot}1$, the function given by equation (4′) becomes independent of K; by expanding the equation in a Maclaurin series, one gets, for small values of K—

$$\frac{k_a}{k} = -\frac{3}{2} \times \frac{2{\cdot}3}{Kkd \times 2{\cdot}3} \quad [-K(1-\theta)] = \frac{3}{2} \times \frac{(1-\theta)}{kd}, \qquad (8)$$

and equation (4) then becomes—

$$E_a = \log\left(\frac{I_0}{I}\right) = -\frac{3}{2} \times \frac{m}{\rho S d \times 2{\cdot}3} \times (1-\theta). \qquad (9)$$

This formula was obtained by Otvis, Stone and Harp[12] in a more rigorous way.

Now, let us compare the experimental results obtained for calcite with these formulae; the results obtained for the four bands have been transformed in (k_a/k), and it is seen that all the experimental points lie between two theoretical curves, one corresponding to $K = 0{\cdot}8$ and one corresponding to $K \leqslant 0{\cdot}1$. In fact, the experimental results agree remarkably well with the theoretical curve corresponding to cubic particles, but this is perhaps just a coincidence.

A more rigorous calculation taking into account absorption and scattering has been published by Primas and Günthard.[14]

From the analytical point of view, the foregoing calculation shows first that the apparent extinction is proportional to the amount of substance per sq. cm, $(m/\rho S)$, as long as the granulometry is constant (Beer's law applied to a suspension of solid particles); this has been confirmed by experiment (Figs. 8 and 9). However, this conclusion is only valid if the number of particles is sufficiently high.

Secondly, the loss of energy by scattering and the intensity of the absorption bands depend to a very great extent on the dimensions of the particles. It is possible to show that the limiting value of the extinction is practically attained (to within 1 per cent.) when $kd \leqslant 0{\cdot}065$; this requires, for an intense band, particles whose diameters are below $0{\cdot}1\ \mu$. Such a fine dispersion is often impossible to achieve in practice, and in analytical problems one is usually compelled to devise methods of dispersion that can be relied on to give reproducible results.

Chemical factors

From the point of view of reproducibility and precision in the quantitative analysis of solid substances, the potassium bromide pellet technique is often better than the Nujol method; however, the act of dispersing a substance in an alkali halide can sometimes produce chemical and physico-chemical interaction between the substance and the dispersing medium, ranging from simple adsorption to a purely chemical reaction that sometimes considerably alters the spectrum. This point is illustrated by the following examples.

Adsorption of the substance on the particles of the dispersing medium—

Farmer[15] observed changes in the spectra of phenols and organic acids induced by grinding with alkali halides; benzoic acid, for example (Fig. 10), showed a change from the spectrum obtained in Nujol that depended very much on the nature of the alkali used: in potassium iodide the spectrum is, as in Nujol, essentially that of the normal dimeric crystalline form, whereas in potassium bromide, potassium chloride and sodium chloride there are progressive changes. It is noteworthy that the two last-named spectra were recorded with a sample that, after grinding, had been heated at 80° C for 2 hours before being pressed in order to sublime the excess of dimer.

The spectrum recorded in sodium chloride is closely analogous to that of benzoic acid in dioxan solution when carboxylic acids are present as monomers, as they associate more readily with solvent molecules than with their own species. This strongly suggests that the spectrum recorded with the sodium chloride disc must be ascribed to monomeric molecules of benzoic acid that have been adsorbed on the sodium chloride particles, probably being linked by hydrogen bonds between the hydroxyl groups and the chloride ions on the crystal surface.

This interpretation has been proposed by Farmer on the basis of a detailed study of the individual spectra: for example, the strong band of the dimer at $7{\cdot}73\ \mu$ due to the coupled C–O stretching and OH planar deformation vibrations is displaced to longer wavelengths in

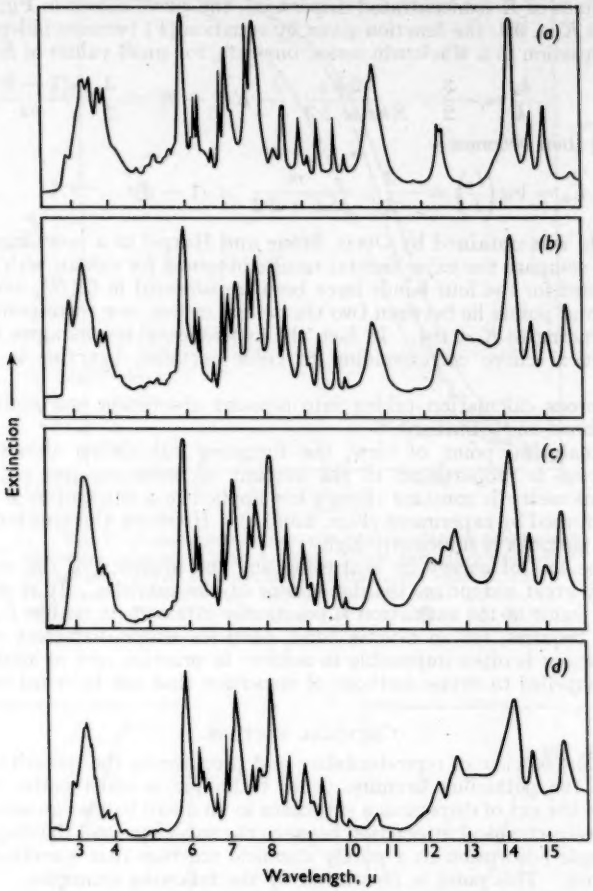

Fig. 10. Spectra of benzoic acid in (*a*) potassium iodide, (*b*) potassium bromide, (*c*) potassium chloride and (*d*) sodium chloride (after Farmer[15])

the order: chloride; bromide; iodide, until, in potassium iodide, it lies close to the corresponding bands for the free monomer near 8·20 μ, which can be explained by the weakening of the hydrogen bond expected with increasing ionic radius.

	Dimer	NaCl	KCl	KBr	KI	Monomer	Dioxan solution
Wavelength, μ ..	7·73	8·04	8·05	8·12	8·21	8·22	8·19

I must add that Tolk and van der Molen[16] observed that the spectrum of benzoic acid in potassium chloride (or bromide or iodide) changes with time: when recorded immediately after pressing, the results that they obtained were the same as Farmer's, but after 3 days, the sample recovered and the spectrum was again that of the dimer; the recovery is more rapid in potassium bromide and potassium iodide than in potassium chloride.

Finally, Karagounis and Peter[17] obtained results rather similar to Farmer's by using the following technique: benzoic acid dissolved in ether was mixed with finely powdered silver iodide; after evaporating the ether, the substance was heated for some time at a temperature a little above that required to fuse the substance and was then pressed into a pellet (Fig. 11). Although for quantities of benzoic acid sufficient to give more than one layer, they

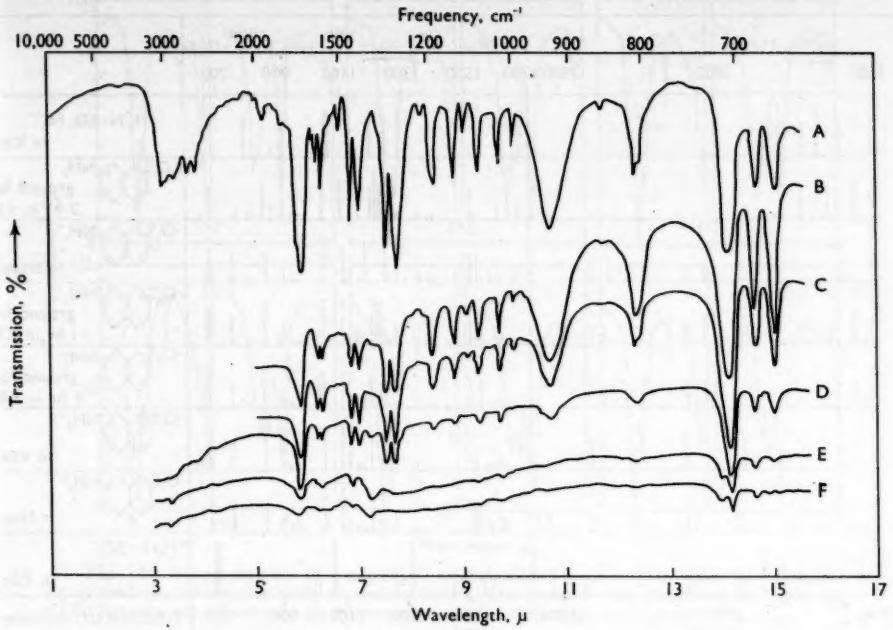

Fig. 11. Spectra of benzoic acid on silver iodide: curve A, solid benzoic acid; curve B, 3×10^{-3}; curve C, 1×10^{-3}; curve D, 4×10^{-4}; curve E, 2×10^{-4}; curve F, 1×10^{-4} g of benzoic acid per g of silver iodide.

obtained the spectrum of the dimer, they observed, on reducing the amount of benzoic acid, that certain bands disappeared more rapidly than others and that some new bands appeared. Their observations agree in general with Farmer's, but Karagounis and Peter are of the opinion that the changes observed in the spectrum of benzoic acid are due to the polarisation of the dimer molecules adsorbed on the surface of the crystal by the electric field of the ions.

CHEMICAL REACTION BETWEEN THE SUBSTANCE AND THE ALKALI HALIDE—

The close contact during grinding, vibrating and pressing between the dispersing medium and a substance with an ionic character (salt or acid) may give rise at the surface to an ion-exchange reaction—

$$XR + \mathrm{KBr} \rightarrow \mathrm{K}R + X\mathrm{Br}.$$

It is easy to understand that in the case when $X \equiv \mathrm{H}$, the liberation of hydrobromic acid, for example, will promote the completion of the reaction.

For instance, grinding a mixture of potassium bromide and benzoic acid in a ball mixer for 20 hours leads to the complete transformation of the acid into potassium benzoate. Heating the mixture for 2 hours in a closed vessel also produces a certain amount of benzoate. We observed this phenomenon even better in the course of a study of the infra-red spectra of aminosulphonic derivatives of naphthalene; the reaction was especially pronounced in the case of 2-aminonaphthalene-7-sulphonic acid (Fig. 12). The spectra of the acid in Nujol and in potassium bromide prepared by mixing for a short time are identical, and their interpretation reveals the zwitterion structure of the substance—

(these should be compared with the spectra of sulphamic acid[18]).

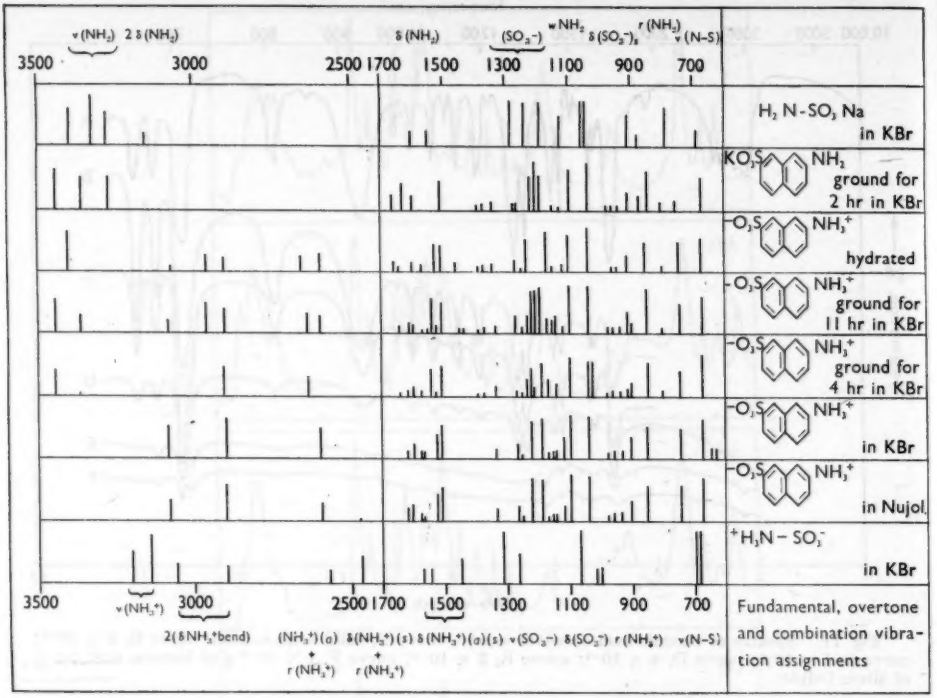

Fig. 12. Interpretation of spectra to show zwitterion stucture

It should be added that the aminosulphonic compound may crystallise with one molecule of water, and this makes some changes in the infra-red spectrum; *e.g.*, splitting of the NH_3^+ band near 2500 cm^{-1}, shifting of the $\nu(SO_3^-)$ bands and introduction of new bands in the region of the deformation vibration of NH_3^+.

Now, by extended grinding of the acid with potassium bromide one produces, and can observe by studying the spectrum, a progressive hydration of the acid and the beginning of the ion-exchange reaction; bands near 3400, 1630 and 1205 cm^{-1} appear, indicating the formation of NH_2. The splitting of the 2600 cm^{-1} band and the appearance of bands at 1170 and 1235 cm^{-1} indicate that some hydrated acid is formed.

POLYMORPHISM—

It is well known that significant differences exist between the spectra of polymorphic forms of the same compound; grinding or pelleting may promote a polymorphic transition to a more stable state. Farmer observed that the grinding of the β form of cinnamic acid with potassium iodide gave the α form; even vibrating for 1 minute is sufficient to produce a spectrum in which the transition is demonstrable, and the transition is complete after grinding for 4 minutes.

Baker[19] gives a good example of polymorphism for α-naphthalene acetamide. He showed that structure I of the substance (typified by the spectrum in Nujol) is progressively converted into structure II with increasing time of grinding: grinding for 10 seconds with potassium iodide in a vibrator is sufficient to produce some transformation; after 120 seconds, the conversion is complete. The same spectrum of structure II could be obtained by re-crystallisation of the substance at room temperature from ethanol or pyridine (Fig. 13).

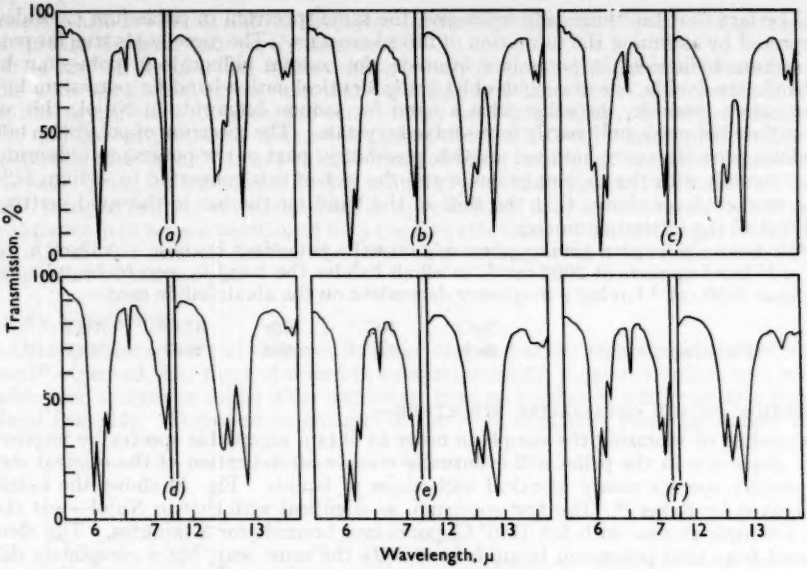

Fig. 13. Polymorphism of α-naphthalene acetamide: spectra of samples (*a*) in Nujol, (*b*) ground for 10 seconds with potassium iodide, and ground with potassium bromide for (*c*) 20 seconds, (*d*) 40 seconds, (*e*) 90 seconds and (*f*) 120 seconds (after Baker[19])

MIXED-CRYSTAL FORMATION—

Ketelaar, Haas and van der Elsken[20] have recorded the spectra of various bifluorides in alkali halide discs and compared them with the spectra recorded for the corresponding substances in Nujol. The bifluoride ion, HF_2^-, gives rise to two vibrations in the infra-red region; the antisymmetric stretching vibration, ν_3, near 1500 cm^{-1} and the deformation vibration, ν_1, near 1200 cm^{-1}; in Nujol mull, ν_3 gives rise to a very broad band between 1400 and 1600 cm^{-1}. They observed in the spectra of the discs not only a sharpening of the bands but also a shift, which can be very important (ν_3 for potassium bifluoride is at 1455 cm^{-1} in Nujol, but at 1599 cm^{-1} in sodium bromide), and sometimes a splitting (see Table I).

TABLE I

ABSORPTION BANDS OF THE BIFLUORIDE ION (HF_2^-) IN VARIOUS DISPERSION MEDIA

Salt	Band for bifluoride ion dispersed in— Nujol, cm^{-1}	potassium chloride, cm^{-1}	potassium bromide, cm^{-1}	potassium iodide, cm^{-1}	sodium bromide, cm^{-1}
Sodium bifluoride ..	1212	1257 (M)	1214	1214	1213
			1258 (M)		1247 (M)
	1600*	1570 (M)	1528 (M)	1600*	1599 (M)
			1650*		1600*
Potassium bifluoride ..	1230	1257 (M)	1257 (M)	1238 (M)	1211 (R)
					1247 (M)
	1455*	1570 (M)	1527 (M)	1475 (M)	1599 (M)
					1600*
Ammonium bifluoride ..	1208	1257 (M)	1258 (M)	1239 (M)	1215 (R)
				1259	1247 (M)
	1600*	1570 (M)	1527 (M)	1478 (M)	1598 (M)
				1600*	1600* (R)

* Very broad. (M) = Mixed crystals. (R) = Reaction with halide.
(Taken from Ketelaar, Haas and van der Elsken.[20])

The fact that the three bifluorides give the same spectrum in potassium chloride can be interpreted by assuming the formation of mixed crystals. The same holds true for potassium and sodium bifluorides in potassium bromide; for sodium bifluoride in potassium bromide the bands are double, one of each doublet being identical with a band for potassium bifluoride in potassium bromide, the other with a band for sodium bifluoride in Nujol; this suggests that sodium bifluoride only partly forms mixed crystals. The spectrum of potassium bifluoride in sodium bromide exemplifies yet another possibility: part of the potassium bifluoride forms mixed crystals with the sodium bromide and the rest of it is converted to sodium bifluoride. These workers have shown that the shift of the band for the ion in the solid matrix halide is related to the lattice parameter.

We have observed a similar phenomenon with potassium cyanide. Although in Nujol the C≡N band appears at 2092 cm^{-1}, in alkali halides the band is seen to be split, the new peak near 2050 cm^{-1} having a frequency dependent on the alkali halide used—

	NaCl	KCl	KBr	RbBr	RbCl
Frequency (ν), cm^{-1} ..	2065	2047	2045	2043	2043

DISTORTION OF THE CRYSTALLINE STRUCTURE—

Grinding or vibrating the sample in order to obtain acceptable spectra by improvement of the dispersion in the pellet will eventually remove all indication of the original structure and produce spectra nearly identical with those of liquids. Fig. 14 shows the example of benzil given by Baker.[19] The first spectrum, *a*—identical with that in Nujol—was obtained from a sample ground with hot (110° C) potassium bromide for 2 minutes. The second, *b*, prepared from cold potassium bromide in exactly the same way, has a completely different band structure and is markedly similar to the spectrum from a 5 per cent. solution in carbon disulphide (spectrum *d*); this means that the particles in pellet *b* are in an amorphous state. Pellet *c* was prepared from cold potassium bromide by using a plastic hammer rod, and the

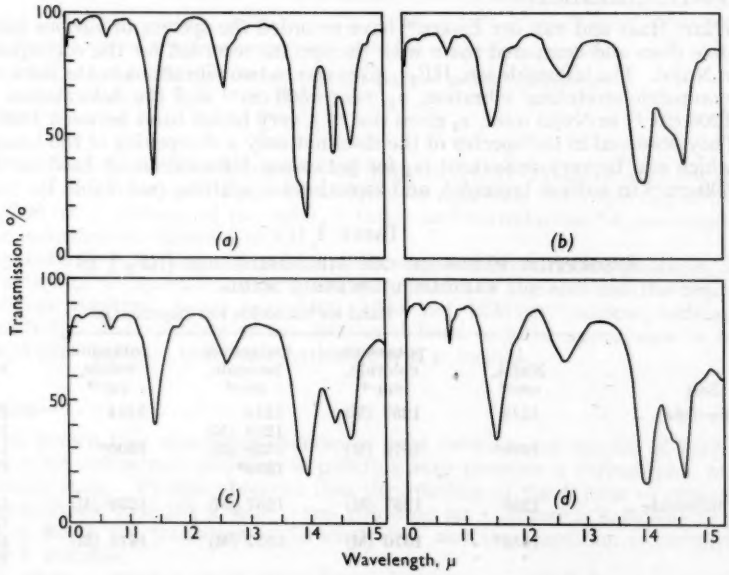

Fig. 14. Distortion of the crystalline structure of benzil: spectra of samples (*a*) vibrated for 2 minutes with hot potassium bromide, (*b*) vibrated with a steel rod for 2 minutes with cold potassium bromide and (*c*) vibrated with a plastic rod for 2 minutes with cold potassium bromide; (*d*) spectrum of a 5 per cent. solution in carbon disulphide (after Baker[19])

spectrum, which is intermediate in character between spectra *a* and *b*, shows that the crystalline structure has been only partly destroyed.

It is easy to see that the factors that play a role in crystal distortion during grinding, mixing with the dispersing medium and pelleting, and hence affect the spectrum, are: the crystal energy of the substance, the total energy used in grinding, the lattice energy of the matrix and the temperature. Stress relaxation, which is shown by a return from a spectrum characteristic of a "liquid" to one characteristic of the crystalline state, is frequently observed as the pellet ages; for substances having a high crystal energy, recrystallisation will proceed rapidly, being so fast in some compounds as to be difficult to observe; for low crystal energies, the substance may remain unchanged for a considerable time. Similar changes can be brought about in a shorter time by heating the pellet in a vacuum oven at a temperature below the melting-point of the sample.

COMPLEX FORMATION—

Although the spectra of thiourea in Nujol and in sodium chloride pellets are identical, Stewart[21] observed that those obtained in caesium bromide, potassium iodide and potassium bromide (and sodium bromide) differ somewhat from each other and differ greatly from those in Nujol (Fig. 15). There are no changes in the N–H stretching vibration bands, but only

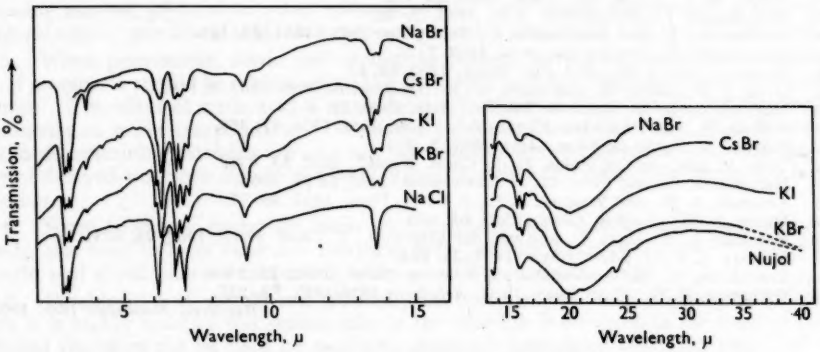

Fig. 15. Spectra of thiourea dispersed in various alkali halides (after Stewart[21])

frequency shifts and band splitting. This behaviour would be expected if foreign particles were introduced into the thiourea lattice, perturbing the molecular vibrations and reducing the four-fold degeneracy of the unit cell. The explanation of complex formation between thiourea and some alkali halides was proposed by Stewart on the basis of the following arguments—

(*a*) Rosenheim and Loewenstamm[22] have given evidence of the interaction of thiourea with potassium iodide, caesium bromide, sodium bromide, potassium bromide and sodium iodide in aqueous solution; analysis of the crystals formed in alcoholc orresponds with the formula: $KBr[(NH_2)C{=}S]_4$.

(*b*) When thiourea and the alkali halide were crystallised from solutions in water or methanol and the resulting material was suspended in Nujol, the spectra were identical with those obtained in pellets of caesium bromide, potassium iodide and potassium bromide.

(*c*) The X-ray diffraction pattern obtained for a powdered sample of the potassium bromide - thiourea complex shows new lines in addition to those of unreacted thiourea and potassium bromide.

(*d*) The formation, in the pellet technique, of complexes between the solid alkali halide and thiourea is observed only with the weaker lattices (potassium bromide and iodide and caesium bromide) and not with sodium bromide and iodide, and, further, the reaction takes place not during the mixing or the grinding but during the pelleting

under pressure. Only the weaker lattices react under pressure, indicating that a mechanism of lattice rupture followed by rapid diffusion is involved. In fact, Bridgman[23] has found a polymorphic transition in thiourea at room temperature for a pressure lying in the range encountered in pellet production, and it is well known that the rate of reaction between solid substances is enormously enhanced at the transition point of one of the partners.

I would like to conclude by saying with Baker that when the potassium bromide pressed-pellet technique was introduced in 1952 to the field of infra-red spectroscopy, it was received with considerable enthusiasm: it solved a number of difficult sampling problems and gave promise of being suitable for quantitative analysis; it seems now, after several years' experience, that there are some occasions when serious difficulties arise that must not be under-estimated.

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Analytical Methods Committee

REPORT PREPARED BY THE METALLIC IMPURITIES IN ORGANIC MATTER SUB-COMMITTEE

Notes on Perchloric Acid and its Handling in Analytical Work

THE Analytical Methods Committee has received the following report from its Metallic Impurities in Organic Matter Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

Report

When the Metallic Impurities in Organic Matter Sub-Committee was re-organised in 1955, it was realised that one of its first duties would be the recommendation of different methods for the destruction of organic matter. It was immediately apparent that the use of perchloric acid is becoming increasingly important, but that it has been neglected in many chemical laboratories owing to common misconceptions about the hazards attendant on its use. Smith[1] has stated, and many chemists agree, that perchloric acid can be used with safety, so long as the user possesses a knowledge of its properties and applies the simple precautions that are appropriate. Much is now known of the properties and the appropriate precautions,

and the following notes have been prepared as a guide. Also included is information about the construction and care of fume cupboards in which the acid is used.

Properties and uses of perchloric acid

1. Perchloric acid is completely stable under ordinary storage conditions in concentrations of less than 85 per cent. The concentrations normally supplied are 60 and 72 per cent.

2. The azeotropic mixture with water contains 72·5 per cent. of perchloric acid and boils at 203° C at 760 mm pressure, so that the evaporation of an aqueous solution of perchloric acid can never produce an acid of dangerously high concentration. If, however, metallic salts are present, the mixture should not be evaporated to dryness over an open flame.

3. Perchloric acid vapour and inflammable gases form violently explosive mixtures, and care should be taken to avoid their formation.

4. Hot 60 to 72 per cent. perchloric acid is a powerful oxidising agent and oxidises all forms of organic matter, but it loses its oxidising properties entirely when cooled and diluted with water. The constitution and properties of any material must be taken into consideration before treatment with perchloric acid, whether the material be vegetable or animal matter or a pure chemical. Generally, the speed of the reaction can be readily controlled, but samples containing alcohol, glycerol or other substances that form esters should not be heated with perchloric acid or perchloric acid mixtures, except under previously well tried conditions.

5. When permissible, nitric acid should be present during an oxidation by perchloric acid; if possible it should be added before the perchloric acid and definitely before evaporation to fumes. The effect of nitric acid is to moderate the reaction by oxidising the more reactive components at lower temperatures. It should be remembered, however, that heterocyclic substances containing nitrogen are as a rule not readily oxidised by nitric acid, and the possibility of delayed reactions should not be overlooked; other procedures are necessary for their oxidation.

6. Some inorganic materials, such as hypophosphites and tervalent antimony compounds, also tend to form explosive mixtures with perchloric acid when hot. A large excess of nitric acid should always be present when oxidising inorganic salts.

7. Perchloric acid should not normally be used for oxidising organic materials with which it is highly reactive but immiscible, as the reaction is localised in the zone of contact. It should therefore not be used for oxidising materials containing much fat until any excess of fat has been removed, since local over-heating cannot be controlled and the temperature may rise dangerously. The oxidation of other substances, such as sulphur, is, however, slower and readily controlled.

8. When perchloric acid is used as a dehydrating agent, as, for example, in the determination of silica, with subsequent filtration, the residue must be thoroughly washed with dilute hydrochloric acid before the filter-paper is ignited.

9. The use of a face shield is an advisable precaution when perchloric acid is being used in laboratory procedures.

Storage and handling of perchloric acid

10. Perchloric acid is supplied, and should be stored, in glass-stoppered bottles. To prevent the possibility of trouble in the event of breakage, perchloric acid should be kept apart from organic chemicals and reducing substances, especially alcohol, glycerol and hypophosphites.

Bottles of perchloric acid should *not* be kept on wooden shelves or benches, since acid trapped in the stopper joints spills over to the benches and these may at some future time become heated and ignite. They should stand in glass or porcelain dishes or on ceramic or other non-inflammable and non-absorbent benches, preferably in such a position that the acid can easily be washed away in the event of breakage or spillage.

11. Any acid spilled should be diluted with water. Swabs used to wipe it up should be, if possible, of wool waste or some other non-inflammable material and not of cotton-wool or other cellulose material.

Perchloric acid that has become discoloured should be diluted with water and washed away.

FUME CUPBOARDS—

The use of fume cupboards constructed entirely of non-inflammable materials has been recommended, and instances have been quoted of wooden fume cupboards taking fire after a long period of use with perchloric acid. On the other hand, many wooden-framed fume cupboards have been in use for perchloric acid evaporation over a period of years without any untoward consequences. The hazard of using wooden fume cupboards will depend on—

(i) the adequacy of ventilation, and

(ii) the exposure of the wood to fumes from digestion vessels.

In a known instance of conflagration, the through-put of perchloric acid was high and the fume cupboard was constructed of unprotected soft wood, which was subjected to considerable heat from an electric hot-plate.

It is suggested that, when a wooden-framed fume cupboard is used—

(*a*) The wood should be teak.

(*b*) The cupboard should have an efficient draught to prevent condensation of the acid vapours.

(*c*) The fans should be left on for a while after digestions have been completed in order to clear residual fumes.

(*d*) The woodwork should be washed down with water at frequent intervals and should be treated occasionally with paraffin wax.

(*e*) The wood should be examined occasionally. A splinter placed on a hot-plate will indicate whether or not the wood has become unduly inflammable.

A satisfactory hood can be constructed from epoxy-resin - glass fibre laminates, but before any particular material is used a small fragment should be treated with hot perchloric acid and its resistant properties confirmed.

Asbestos cement or vitreous tiles provide the most suitable material for the bases of fume cupboards and hoods.

Glass fume-eradicators are also available as alternatives to fume cupboards,[1] and their use inside fume cupboards is recommended.[2]

Dirt and dust must not be allowed to accumulate in fume cupboards or fume ducts used for perchloric acid. A fume cupboard in which perchloric acid is used should not be connected to an ordinary chimney flue.

Readily oxidisable cements, such as those made with litharge and glycerine, must not be used to seal cracks in fume cupboards. Pastes of Alundum cement and sodium silicate or similar materials are satisfactory.

The Sub-Committee expresses its gratitude to the members of B.I.S.R.A. Methods of Analysis Committee for their assistance in compiling these notes.

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A Biological Assay for Gibberellic Acid with Rice Seedlings

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A convenient laboratory method for the quantitative bio-assay of gibberellic acid is described. The statistical aspects are discussed.

METHODS for the biological assay of the gibberellins reported in the literature fall into two groups, both involving shoot extension, to measure the amount present. In the first group, young whole plants are supplied with gibberellin and their shoot extensions are compared

with those of controls supplied with standard solutions. The second group includes those methods in which selected sections of the plant shoot are detached and treated *in vitro*, the increase in extension of the sections being compared with that of untreated controls.

In general, methods with the whole plant[1,2,3,4] are the more accurate. Owing to the replication of plants necessary to attain this accuracy, however, these methods require considerable greenhouse space and also the lapse of sufficient time for the gibberellin to show its full effect.

The methods with detached sections of plant[5,6] can be carried out in the laboratory in a shorter time, but are not so accurate.

With the increasing experimental use of gibberellic acid, the need has arisen for an accurate quantitative assay by which residues in food may be determined. (Methods for extracting gibberellin A[7,8] and gibberellic acid like substances[6,9] from plants have been described.) Such an assay should be capable of dealing with large numbers of samples and of being carried out in the laboratory and completed in 2 or 3 days.

The search for a suitable method included the consideration of several species and varieties of plants, mainly cereals, and also certain secondary properties of gibberellic acid, such as its ability to by-pass the light requirement for germination in certain varieties of lettuce seed.[10] It was found that in the variety "Trocadero" a strong tendency for light requirement existed in fresh seed (germination being 6 to 10 per cent. in darkness and 70 to 73 per cent. in light) and there was a relationship between gibberellic acid concentration and the degree of release from this requirement. This method was abandoned because the seed, even though stored in the dark, fairly rapidly lost this characteristic.

Although several of the plant subjects examined appeared promising, and could probably be used as assay material, the most consistent results were obtained from young rice seedlings. The variety of rice used in all these experiments, "Precoce Rossi," was obtained from Italy. No claim is made that this is the best variety to use, and other varieties may indeed give improved results, but this one was found satisfactory.

All these experiments were carried out with gibberellic acid, but the method could probably be used for other gibberellins.

Experimental

The most convenient method of applying gibberellic acid to young rice seedlings was found to be by the root, and the assay method described was devised with this treatment.

Preliminary work on assays with rice seedlings showed that much of the accuracy of the test depends on the size of the seedlings when first treated; it was found that the ideal seedling size was 2 to 5 mm for the coleoptile and 10 to 15 mm for the root (see p. 220, notes on experiment No. 6). To obtain approximately 25 per cent. of ideal-sized seedlings, rice seeds were soaked for 6 to 8 hours in tap-water before placing them in Petri dishes containing acid-treated sterile sand saturated with tap-water. The seeds were embedded in the sand, so that the coleoptile emerged on the upper side of the seed. The prepared Petri dishes were incubated for 72 hours at 28° C. Other temperatures were tried, but all resulted in rather fewer ideal-sized seedlings after a 72-hour incubation period.

Various methods were tried to ensure that the seedlings remained upright during the test, including the use of waxed cardboard moulded with grooves to hold the seedlings in position and punched with holes that allowed the roots of the seedlings to dip into the treatment solution in a Petri dish below the cardboard. This method proved unsatisfactory, as the wax tended to soften during incubation and a considerable amount of "run over" of solution was encountered. Because of this, the apparatus described below was devised; it proved to be much more satisfactory in practice and was adopted in all further tests. It consisted of a rectangular glass vessel (8·5 cm × 2 cm × 1 cm, internal dimensions) over which was fitted a cover of stiff aluminium foil (gauge $^{3}/_{1000}$ inch) moulded with a trough running along the middle, parallel with and equidistant from the longer sides of the vessel. Six holes, 4 mm in diameter, were punched in the base of this trough. The depth and breadth of the trough and the size of the holes (all 4 mm) were just sufficient to allow the rice seedlings to stand upright with roots through the holes and coleoptile above the level of the sides of the trough. To each dish 8 ml of test solution were added, this being sufficient to immerse completely the roots of the seedlings without undue risk of spilling. As a safeguard against any "run over" of solution during the test, a film of petroleum jelly was spread on the upper edges of the glass vessels before fitting them with the aluminium-foil covers.

The prepared dishes were transferred to a tray, randomised, placed in a polythene humidity chamber, which maintained the humidity at above 90 per cent., and incubated at 29° to 30° C with illumination of 6 foot-candles for 72 hours. Readings of the shoot height were taken after incubation for 24, 48 and 72 hours. All seedlings showing abnormal growth, due either to fungal attack or deformity, were removed before taking readings.

The low level of illumination previously mentioned calls for further explanation. During the early experiments, the maximum illumination attainable within the humidity chamber in a temperature-controlled room was 6 foot-candles. For consistency, the remaining experiments were carried out under the same conditions, but some were carried out at 16 and 35 foot-candles. The results suggested that at least as good and possibly better assays could be obtained by increasing the illumination.

All test dilutions of gibberellic acid were made with tap-water.

Results

Experiments were carried out to find the most suitable apparatus and technique for handling the rice seedlings, to establish the dose - response pattern and to improve the accuracy of the method; the results are shown in Table I.

Table I

Average plant height

Each result in experiments 1 and 2 is the mean of 2 determinations
Each result in experiments 3 and 7 is the mean of 8 determinations
Each result in experiments 4, 5 and 6 is the mean of 6 determinations

Gibberellic acid dose, µg per ml	Shoot height 24-hour reading, mm	Shoot height 48-hour reading, mm	Shoot height 72-hour reading, mm	Shoot height 24-hour reading, mm	Shoot height 48-hour reading, mm	Shoot height 72-hour reading, mm	Shoot height 24-hour reading, mm	Shoot height 48-hour reading, mm	Shoot height 72-hour reading, mm
Experiment No. 1—				*Experiment No. 2—*			*Experiment No. 3—*		
Control	9·30	25·65	34·80	8·30	21·25	36·05	9·50	21·60	43·66
0·1	9·70	26·20	34·85	8·60	21·70	37·35	9·51	20·84	43·53
0·5	10·40	28·00	38·35	9·10	24·10	39·10	10·09	24·43	49·39
1·0	9·80	28·35	39·05	9·05	26·15	45·40	10·93	27·06	55·81
2·0	—	—	—	9·60	27·40	46·95	—	—	—
3·0	—	—	—	9·90	26·15	46·50	—	—	—
4·0	—	—	—	9·55	24·75	45·05	—	—	—
5·0	10·85	32·05	42·80	9·45	25·15	43·65	11·61	29·36	63·38
7·0	—	—	—	9·95	26·95	47·30	—	—	—
10·0	12·10	34·10	45·70	9·05	27·65	47·85	11·10	27·41	56·34
Experiment No. 4—				*Experiment No. 5—*			*Experiment No. 6 (small seedlings—*		
Control	10·32	25·57	43·55	—	—	—	—	—	—
0·25	12·72	29·33	50·13	—	—	—	—	—	—
0·4	—	—	—	9·63	24·95	46·30	—	—	—
0·5	13·08	31·37	54·38	9·78	25·90	47·85	8·05	23·33	42·15
0·6	—	—	—	9·65	26·15	49·10	—	—	—
0·8	—	—	—	9·90	26·48	49·62	—	—	—
1·0	12·73	32·55	57·23	10·47	27·92	51·88	8·77	25·83	47·15
1·2	—	—	—	10·43	28·08	52·42	—	—	—
1·6	—	—	—	10·98	29·18	55·15	—	—	—
2·0	12·70	33·38	60·08	10·45	29·07	55·73	8·57	26·48	48·02
2·4	—	—	—	10·37	29·45	55·33	—	—	—
4·0	13·90	36·57	65·18	—	—	—	—	—	—
8·0	13·38	34·98	63·15	—	—	—	—	—	—
Experiment No. 6 (medium seedlings)—				*Experiment No. 6 (large seedlings)—*			*Experiment No. 7—*		
Control	—	—	—	—	—	—	7·55	24·34	41·67
0·25	—	—	—	—	—	—	8·95	27·55	47·13
0·5	10·20	27·57	49·08	13·58	33·62	57·37	9·05	29·00	50·75
1·0	10·90	29·52	53·60	13·80	34·67	61·98	9·40	30·06	53·30
2·0	11·03	32·10	58·75	13·52	35·60	65·10	10·19	32·35	57·48
4·0	—	—	—	—	—	—	9·96	32·33	59·23
8·0	—	—	—	—	—	—	10·56	32·83	61·43

Experiments Nos. 1 and 2, in which a waxed-cardboard holder was used, suggested that, despite the defects of this type of apparatus, a linear relationship existed between log (dose) and a suitable response metameter (see below) for gibberellic acid concentrations between 0·1 μg per ml and 5 to 10 μg per ml.

During these early experiments a choice was made as to which of the three readings (at 24 hours, 48 hours and 72 hours), or which combination of them, gave the most information; the original readings, their logarithms, differences between the readings and sums of them were all studied. The criteria adopted in making this choice were (*a*) that there should be no significant departure from linearity in the mid-range of doses chosen from the results, and (*b*) that the highest slope to error ratio should be sought. By these criteria, the best choice was either the 72-hour reading or the 72-hour reading minus the 24-hour reading. As these were equally informative, the former was chosen for convenience. Although subsequent comments refer specifically to the 72-hour reading, they are usually applicable to the others (see "Response Curve," p. 220).

Experiments Nos. 3, 4, 5, 6 and 7 were carried out with aluminium-foil holders and were designed to supplement the earlier results by determining more precisely the nature of the dose - response relationship. Experiment No. 3, in which replication was increased, confirmed the linear response between 0·1 and 5·0 μg of gibberellic acid per ml, but the value for 10 μg per ml was considerably lower than that for 5 μg per ml.

In experiment No. 4 the value for 4 μg per ml, although not departing significantly from linearity, was somewhat high. This led to an examination of the effect of seedling size on response, and the result suggested that seedling allocation had not been completely random.

Experiment No. 5 was conducted as a trial assay, and great care was taken over the selection of seedlings. Three samples of crystalline gibberellic acid were weighed out, one being used as a standard, the others as "unknowns." The potency of unknown A was 120 per cent. of the standard and that of unknown B was 80 per cent.; the levels of each used were 0·5, 1·0 and 2·0 μg per ml. Positional effects were examined in two 3 × 3 Latin squares, each plot being split into 3 sub-plots, 2 degrees of freedom for interaction being confounded in each square, this being the most suitable design for nine treatments in the laboratory space available. For potency estimation, within-plot information only was used. The three samples gave parallel linear responses. Table II shows the totals of the six determinations of the 72-hour shoot heights for each of the nine samples. The complete results are shown in Table I.

TABLE II

TOTALS USED IN CALCULATION OF POTENCY

Each result is the total of six determinations

	Sum of 72-hour shoot heights—			
	at low dose-level (0·5 μg per ml), mm	at medium dose-level (1·0 μg per ml), mm	at high dose-level (2·0 μg per ml), mm	Total, mm
Standard	287·1	311·3	334·4	932·8
Unknown A	294·6	314·5	332·0	941·1
Unknown B	277·8	297·7	330·9	906·4
Total	859·5	923·5	997·3	2780·3

The potency, P, of the unknowns tested at three dose-levels and dilution ratio ×2 is given by the expression—

$$P = 100 \times \text{antilog}\left[\frac{0{\cdot}60206 \times (\text{Mean response of unknown} - \text{Mean response of standard})}{(\text{Mean response at high dose-level} - \text{Mean response at low dose-level})}\right].$$

As the replication on substances and doses is the same, *i.e.*, 18, totals can be substituted for averages, hence—

$$P_A = 100 \times \text{antilog}\left[\frac{0{\cdot}60206\ (941{\cdot}1 - 932{\cdot}8)}{(997{\cdot}3 - 859{\cdot}5)}\right] = 109 \text{ per cent.},$$

similarly, $P_B = 77$ per cent.

Precision of assay

The coefficient of variation of potency of such an assay is—

$$2\sqrt{\frac{\text{Replication on levels}}{\text{Replication on samples}}} \times \sqrt{\frac{\text{Error mean square}}{\text{Slope mean square}}} \times \log_e 10 \times \log_{10} (\text{dilution ratio}).$$

With equal replication on levels and standards and a dilution ratio of 2, this gives a coefficient of variation of $138{\cdot}6 \times \sqrt{\frac{\text{Error mean square}}{\text{Slope mean square}}}$.

In experiment No. 5 the coefficient of variation is 16·9.

Experiment No. 7, if designed as an assay, would have given a coefficient of variation of 13·0.

Experiment No. 6 was designed to investigate the response given by small, medium and large seedlings grown in separate dishes. The small seedlings gave significant curvature and were not used in subsequent work. It was decided also to exclude the large seedlings, as any inequality in their allocation could introduce considerable bias. As a result of this experiment the ideal size of seedlings previously mentioned was fixed.

Experiment No. 7 was carried out several months after the beginning of this work to establish whether or not the seed sample had altered physiologically with time and had thereby perhaps affected the dose - response relationship. No significant departure from linearity could be detected in the range 0·25 to 8·0 μg of gibberellic acid per ml.

Discussion of results

The biological assay of a plant growth-promoting substance, such as gibberellic acid, involves certain problems that affect other types of biological assay to a lesser extent. These were considered in the choice and development of the assay and in the interpretation of the results.

Test organism—

As Brian, Elson, Hemming and Radley[11] have shown that gibberellic acid has no effect on micro-organisms of various types, the test organism was looked for among higher plants, where the main biological action of interest lies. Whereas strains of micro-organisms can be maintained in a constant condition with relative ease, it is more difficult to do this with higher plants. Further, individual variation cannot be ignored, as it can in assays with micro-organisms in which a large number of individuals are present.

Such difficulties become obvious when percentage germination is the response measured, the maturity of seeds within a batch and physiological changes with time often leading to increase in variability. These facts precluded the use of lettuce seed for a gibberellic acid assay (see p. 217). By using shoot extension of a higher plant, some of these difficulties can be overcome, provided that careful initial selection of seedlings is made. The control of humidity, light and temperature presents little difficulty when the assay organism is small.

Response curve—

Shoot height, chosen as the response, is liable to anomalous results, which can be minimised by initial selection. Other anomalous values can be eliminated only when the cause is obvious, such as diseased seedlings or drying-out of dishes. In addition, if the variation within dishes is shown to be reasonably uniform, outliers can be shown up by a statistical test such as that described by Dixon,[12] which must be applied to the whole of the data and not merely to selected results.

A range of doses was chosen within which a linear relationship existed. Four relationships were investigated for linearity, namely, response and log(response) as functions of dose and log(dose). When dose was used as the dose metameter, a curve was obtained. Within the limits of error variation, linearity could be obtained with log(dose). As it proved immaterial (since, within a narrow range, $\log(1 + x) = x$, for small values of x) whether response or log(response) was used as response metameter, the former was chosen for simplicity. The linear relationship was consistent in the middle range, but tended to vary

at the extremes on some occasions. Which was the most suitable of the three readings (that at 24, 48 or 72 hours) depended on the effect of time on the error variation and on the slope. In this work, the 72-hour reading was found to give the highest slope to error ratio, at the same time maintaining linearity.

ERROR VARIATION—

Although the standard error increased with time, as also did the slope and average shoot height, there was no evidence of its being affected by the gibberellic acid dose-level. Hence, a simple untransformed analysis of variance was performed.

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The Determination of Chloroform in Aqueous Pharmaceutical Preparations

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The usefulness of a gas-chromatographic procedure for determining chloroform in a variety of pharmaceutical preparations is shown, and a special injection system for dealing with non-volatile materials is described. A calibration method in which peak heights are used is shown to be adequate for this application of gas chromatography.

Recovery experiments and comparisons with chemical determinations show that results are reliable. The method is rapid; an assay can be performed in 15 minutes.

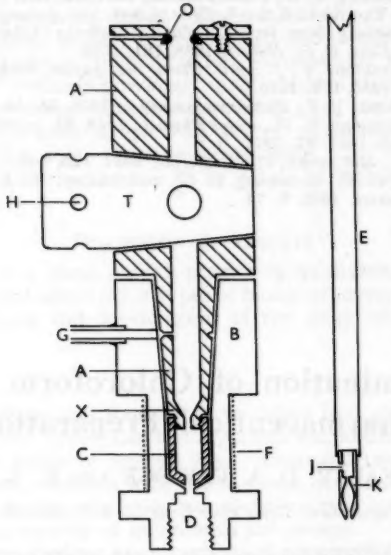
MANY aqueous pharmaceutical preparations contain chloroform as a flavouring or preservative in concentrations between 0·1 and 0·5 per cent. and certain other preparations, such as Tincture of Chloroform and Morphine, contain up to 15 per cent. Chemical methods at present in use for determining chloroform in such samples are time-consuming, and, if many assays have to be carried out for control purposes, the time factor is important. By using gas chromatography, it has been possible to develop a method of determining chloroform that takes only 15 minutes.

In devising this method it was considered that no pre-treatment of the samples by way of distillation or solvent extraction could be tolerated. Most of the samples contain non-volatile solid matter, and, if these were placed directly into conventional gas-chromatography equipment, the column would quickly become blocked and require frequent changing. It was therefore necessary to devise and build a sampling system to overcome this difficulty.

Experimental

Injection system—

The injection system (see Fig. 1) is based on that described by Tenney and Harris,[1] in which the sample is added from a micropipette that can be filled by capillary action. The pipettes, K, are made of glass of about $\frac{3}{16}$ inch diameter and are fixed with Araldite cement into a polished steel rod, E, of $\frac{1}{4}$ inch diameter. These dimensions are such that, whereas the pipette passes freely through the O-ring, O, the steel rod makes a gas-tight seal, so that the stainless-steel tap, T, can then be opened to allow the pipette to be pushed down and its tip located in the cone, X. This delivery tip is ground to seat accurately in the cone so that the gas entering the injection system at G is forced to pass through the hole, J, in the pipette and thus eject the sample. Once the sample has been ejected, the pipette is first withdrawn sufficiently to allow the tap to be closed and is then removed. The opening and closing of the tap are facilitated by a handle that fits into one of four holes, H.



A = Brass block
B = Stainless-steel block
C = Cup
D = Column
E = Polished steel rod
F = Heating element
G = Gas inlet
H = Handle-fixing holes
J = Gas inlet to pipette
K = Pipette
O = O-ring
T = Stainless-steel tap
X = Cone

Fig. 1. Injection system

The sample is ejected from the pipette into a cup, C, which is filled with a suitable material, such as glass-wool, so that the non-volatile components are trapped and prevented from reaching the column, D. When it becomes necessary to change the filling in the cup, the whole of the brass block, A, can be withdrawn from the stainless-steel block, B, into which it fits with a standard conical joint. The cup can then be unscrewed from block A and replaced by another filled cup, the whole operation taking only a short time.

Block B, which is screwed to the lid of a constant-temperature oil-bath, is wound with a heating element, F, designed to maintain the cup at a suitable temperature. The chromatographic column, which screws directly into the injection system at D, is made from $\frac{1}{4}$-inch external diameter copper tubing; it is wound round a thermal-conductivity detector and both are immersed in oil in the constant-temperature bath. The gas supply, which is heated by passage through a spiral tube immersed in the bath, flows through one arm of the thermal-conductivity cell before entering the injection system.

STATIONARY PHASE—

It was considered that the following criteria would represent the ideal conditions obtainable—

(*a*) The chloroform peak must precede the water peak.
(*b*) The chloroform peak must be sharp and reasonably symmetrical if peak heights are to be used for calibration purposes.
(*c*) Because of the time factor, the water must be eluted quickly.
(*d*) It must be possible to resolve chloroform from the lower alcohols, particularly ethanol, because of the presence of the latter in some of the samples.
(*e*) The stationary phase must not be modified by the passage of comparatively large amounts of water.

Little information is available in the literature on the application of gas chromatography to samples containing a high proportion of water. Polyethylene glycol 600,[2] glycerol[3] and triethanolamine[4] have been reported by different workers as being suitable materials for use as stationary phases, but none of them satisfied all the above conditions.

Ray[5] has described a method of removing most of the water from samples before chromatography, and, although his technique could not be applied to samples with a very high water content, the idea of preventing the water from reaching the chromatographic column was tried. The volatilised sample was first passed through a small removable column containing a suitable compound by means of which it was hoped to remove the water and to allow the other volatile constituents to enter the main column. Alumina, magnesium oxide, magnesium perchlorate, silica gel, calcium oxide and sodium carbonate were tried, but none of them retained the water under the operating conditions used. The use of calcium carbide and calcium hydride was also investigated, but although the water was retained the liberated gases interfered considerably when nitrogen was used as the carrier gas. The use of a molecular sieve for retaining the water has also been suggested and would probably be successful, but it was thought that it would be more satisfactory, if possible, to allow all the volatile components of the samples to pass through the column. A high-molecular-weight polyethylene glycol, Carbowax 1500, was eventually found to satisfy all the above-mentioned requirements for a stationary phase.

CALIBRATION—

Because many samples are thick suspensions or syrups, it is difficult to ensure that a standard amount is transferred to the column, and an internal-standard procedure is therefore considered to be essential. *n*-Propanol is used for this purpose, as its peak appears on the chromatograms between those of chloroform and water. Calibration curves are obtained by measuring the ratio of the peak heights of chloroform and *n*-propanol and plotting this ratio against the percentage of chloroform. It is possible to use peak heights for this purpose rather than peak areas, because standard conditions can be maintained and the peaks are sharp and symmetrical.

The results in Table I show the effect of sample size on the ratios of the peak heights of chloroform and *n*-propanol at various concentrations of chloroform. These ratios show that this particular standard functions well as such and that it is not necessary to measure peak areas for this simple application of gas chromatography.

TABLE I

EFFECT OF SAMPLE SIZE ON THE RATIO OF PEAK HEIGHTS OF CHLOROFORM AND *n*-PROPANOL AT DIFFERENT CONCENTRATIONS OF CHLOROFORM

Size of sample, µl	Concentration of chloroform, %	Ratio of peak heights
12	0·1	0·66
25		0·62
36		0·68
12	0·2	1·10
25		1·13
36		1·16
12	0·4	2·33
25		2·34
36		2·34

The results in Table II show the ratios of the peak heights of chloroform and *n*-propanol for three standard solutions, each examined in triplicate. It can be seen that good reproducibility can be obtained between replicate determinations and that a calibration curve of ratio against concentration of chloroform is linear and passes through the origin.

TABLE II

RATIO OF PEAK HEIGHTS OF CHLOROFORM AND *n*-PROPANOL FOR THREE STANDARD SOLUTIONS EACH CONTAINING 1 per cent. OF *n*-PROPANOL

Concentration of chloroform, %	Ratio of peak heights
0·1	0·59, 0·62, 0·59
0·2	1·15, 1·13, 1·14
0·4	2·26, 2·27, 2·30

The calibration curve cannot be reproduced exactly from day to day, but is subject to slight changes in slope. It has, however, proved in practice only necessary to check two points on the calibration curve each day.

METHOD

PROCEDURE—

Standards containing 0·1, 0·2 and 0·4 per cent. of chloroform and 1 per cent. of *n*-propanol in water are prepared. The samples are diluted as necessary to adjust the chloroform content to within the standard range, and 1 per cent. of *n*-propanol is then added. Standards and samples are chromatographed under the following conditons—

Column length—3 feet 6 inches.
Column temperature—88° C.
Stationary phase—20 per cent. of Carbowax 1500 on 36 to 85-mesh Chromosorb.
Nitrogen flow rate—40 ml per minute.
Flash-heater temperature—145° C.
Detector—Thermal-conductivity cell with platinum wires 4 inches long and 0·001 inch thick; nominal resistance 25 ohms, the wires in the two channels being matched to within 0·1 ohm.
Detector current—200 mA.
Recorder—Honeywell-Brown with 2·5-mV full-scale deflection and chart speed of 12 inches per hour.

The column characteristics under these conditions are as follows—

Height equivalent to a theoretical plate—0·087 cm.
Retention volume (*chloroform*)—169 ml.
Retention volume (n-*propanol*)—238 ml.
Time to complete elution of water after injection—20 minutes.

RESULTS

Some recovery experiments have been carried out by examining samples directly and after addition of a known amount of chloroform. The results are shown in Table III.

TABLE III

RECOVERY OF CHLOROFORM FROM PHARMACEUTICAL PREPARATIONS

Sample	Concentration of chloroform found, %	Concentration of chloroform added, %	Total chloroform found, %	Recovery, %
Mixture of ammonium ferric citrate for infants	0·236	0·15	0·390	102
	0·240		0·388	99
Suspension of sulphadiazine	0·096	0·10	0·194	98
Suspension of sulphaguanidine	0·118	0·10	0·222	104

Table IV shows the results obtained on a number of samples compared with those obtained chemically.

TABLE IV

COMPARISON OF RESULTS BY GAS CHROMATOGRAPHY AND BY A CHEMICAL METHOD

Sample	Chloroform found by— chemical method, %	gas chromatography, %
Methylcellulose laxative	0·20	0·18
Proprietary tonic	0·40	0·42
Liquid paraffin and magnesium hydroxide emulsion		
(1)	0·28	0·33
(2)	0·24	0·22
Cough syrups (1)	0·12	0·14
Cough syrups (2)	0·11	0·11
Cough syrups (3)	0·24	0·25
Syrup codeine phosphate (1)	0·16	0·15
Syrup codeine phosphate (2)	0·19	0·18
Tincture of chloroform and morphine (1)	12·1	12·6
Tincture of chloroform and morphine (2)	13·2	13·1
Tincture of chloroform and morphine (3)	12·0	11·9
Cough mixtures (1)	0·57	0·58
Cough mixtures (2)	0·56	0·57
Cough mixtures (3)	0·54	0·57
Cough mixtures (4)	0·25	0·25
Liniment of chloroform	48·3	50·6
Aluminium hydroxide gel (1)	0·18	0·27*
Aluminium hydroxide gel (2)	0·10	0·17*
Aluminium hydroxide gel (3)	0·12	0·26*
Diarrhoea mixture	0·55	0·52
Diuretic concentrate	0·60	0·59

* Because of the large discrepancy between the chemical and the gas-chromatographic results, two standards were prepared containing 0·2 and 0·3 per cent. of chloroform. The following results were obtained—

Concentration of chloroform in standard, %	Chloroform found by— chemical method, %	gas chromatography, %
0·23	0·12	0·22
0·33	0·20	0·33

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The Sampling and Analysis of Toxic Vapours in the Field

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A system is described for multiple sampling of toxic vapours in the field, in which a simple bubbler with critical-orifice control of flow rates is used. An investigation of the factors affecting absorption efficiency is reported. The particular application of the scheme to the field assessment of "nerve gases" is discussed, the analytical method being adapted to permit processing of the samples in the bubbler.

Although the system was developed for studies on the "nerve gases," it has since been successfully applied to field measurements of chlorine, phosgene, cyanogen chloride and mustard gas and it could no doubt be used for many other volatile toxic materials.

Investigations of the distribution of toxic vapours in the atmosphere often call for sampling and analysis over a large area. This may involve considerable effort in equipment and labour, particularly when the methods used are those developed initially for work on a small scale, for which simplicity is less important. A situation such as this arose a few years ago in field studies of the "nerve gases," and a review of the methods in use at the time resulted in the development of a new system, which offered substantial economies without loss in precision.

An account of the work is given here, as the principles outlined may be adaptable to other problems involving vapour sampling and analysis at a large number of points. The system would be directly applicable to the assessment of certain insecticides that can be determined by the colorimetric method used for the "nerve gases." More recently it has been successfully adapted for field sampling and analysis of chlorine, phosgene, cyanogen chloride and mustard gas.

Original procedure—

The analytical method most generally used for the colorimetric determination of the "nerve gases," *e.g.*, Tabun (ethyl N:N-dimethylphosphoramidocyanidate) and Sarin (*iso*-propyl methylphosphonofluoridate), depends on their reaction with hydrogen peroxide and an aromatic base of the benzidine type in alkaline solution.[1,2] This has been the basis of both the original field procedure and the modified system that is the subject of this paper. For some years preceding this work, the standard field method was as follows.

At each sampling point a spined glass bubbler (see Fig. 1) containing 20 ml of *cyclo*-hexanol was connected to an injector operated by compressed air and fitted with a ball-type flowmeter. At the appropriate time, the injector was set to draw air through the bubbler at a rate of 10 litres per minute. After return to the laboratory the samples were analysed in the following stages—

(*i*) The sample was transferred to a measuring cylinder, the bubbler was rinsed with a phosphate buffer (pH 5·5) and the total volume was made up to 40 ml with buffer.
(*ii*) A suitable aliquot was transferred to a 25-ml calibrated flask.
(*iii*) One millilitre of 1 per cent. hydrogen peroxide was added.
(*iv*) One millilitre of 1 per cent. *o*-dianisidine solution was added.
(*v*) Tripotassium phosphate buffer (0·2 *M*) was added to raise the pH to 11·0.
(*vi*) The volume was made up to 25 ml with an acetone - water mixture.
(*vii*) The colour intensity was measured in 3-cm optical cells with an absorptiometer.

General principles—

Considerable experience of the conditions governing the dianisidine - peroxide reaction with "nerve gases" had been gained in the laboratory before this work was undertaken.[2] The general principles laid down to guide development were—

(1) procedure should be such that checking for general functioning can be carried out as far as possible in the laboratory immediately before the equipment is transported to the experimental site;

(2) flow-rates should be controlled by a critical-orifice technique;
(3) laboratory processing should be simplified and the number of transferences undergone by each sample should be reduced as far as possible;
(4) the procedure should permit positive identification of each sample throughout, to avoid confusion of samples when large numbers are being processed;
(5) the equipment should be light and resistant to hazards of transport over rough country. It should be as simple as possible, both to reduce cost and to make possible the replacement of damaged items with ordinary laboratory facilities.

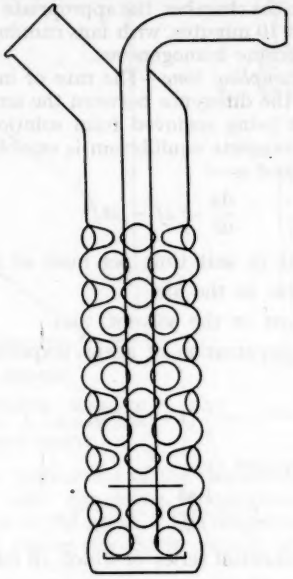

Fig. 1. Spined bubbler

The first step was to specify a flow rate of 1 litre per minute; this was chosen as it could be achieved by critical-orifice control without excessive power consumption, and, by reducing the volume of absorbent, sensitivity should be adequate for most purposes. It was also clear that the use of injectors as sampling pumps would have to be abandoned. They cannot be used conveniently with critical orifices and consequently the control valve has to be set for each instrument after setting up on the experimental site. Accordingly, it was decided to operate the new samplers by electrically driven pumps, various types of which would be suitable; for the development work a double-diaphragm T.1 pump, designed for operating certain bomb-sight computers, was found to be satisfactory.

Another choice that had to be made at an early stage was whether to continue with the use of a bubbler with liquid absorbent or to change to a device containing a solid adsorbent, such as silica gel. The latter had certain advantages, but it was decided to adopt a liquid absorbent when experience showed that loss of sample owing to hydrolysis on the silica gel was taking place.

Experimental

Development of the bubbler—

To avoid loss in sensitivity, it was decided at the outset that the volume ratio of absorbent liquid to air sampled must not be greater than in the existing bubbler. For a flow rate of 1 litre per minute this limited the volume of solvent to 2 ml, and it was evident that the dimensions of the bubbler had to be reduced, although the provision of spines or beads to promote contact between liquid and gas phases would be more difficult on a small scale. Preliminary experiments showed that, with this small volume of liquid in a narrow tube and air drawn in through a small jet, the liquid was carried up the sides of the tube to give a

large surface area and the break-up of the air stream at the jet promoted good mixing. It seemed likely, therefore, that reasonably efficient removal of vapour might be achieved without spines or beads.

The various factors that would determine the efficiency of this method of removal of vapour from an air stream were then examined experimentally with Sarin vapour. The initial experiments were done in the laboratory, but later a 100 cubic metre static chamber was used; this had the advantage that more uniform vapour concentrations could be achieved and it was possible to take larger numbers of samples simultaneously. In order to set up any required nominal concentration in the chamber, the appropriate weight of agent was evaporated in a stream of hot air and at least 10 minutes, with fans running, was allowed after dispersion to permit the distribution to become homogeneous.

Effect of concentration and sampling time—The rate of increase in the concentration of agent in the bubbler is equal to the difference between the amount dissolving in the solvent from the incoming air and that being removed from solution by the effluent air. In an ideal system, *i.e.*, one in which complete equilibrium is established between the vapour and liquid phases, this may be expressed as—

$$\frac{dx}{dt} = cf - sxf \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where f = moles of air sampled in unit time per mole of solvent,

c = mole fraction of agent in the air,

x = mole fraction of agent in the solvent, and

s = saturated vapour concentration of agent (expressed as a mole fraction).

Integration of (1) gives—

$$x = \frac{c}{s}(1 - e^{-fst}) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

or expressed as a percentage recovery (y)—

$$y = \frac{100}{fst}(1 - e^{-fst}) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

This can be expanded as an exponential series of which all but the first two terms may be neglected—

$$y = 100\left(1 - \frac{fst}{2} + \frac{(fst)^2}{6}\right) \quad .. \quad .. \quad .. \quad .. \quad (4)$$

This indicates that percentage recovery should be independent of the concentration of agent in the air. The effect of sampling time on recovery has been calculated for Sarin vapour at 25° C, the air flow rate being 1 litre per minute and 2 ml of *cyclo*hexanol being used as the solvent; the results were as follows—

Sampling time, minutes	1	5	10	15
Recovery, %	99·6	98·0	96·0	94·1

Some experimental results obtained in the static chamber were as follows—

Sampling time, minutes	2	5	10			15
Concentration of Sarin, mg per cubic metre	19	18	2·7	8·2	23·0	17·5
Mean recovery, %	94·5	93·6	92·7	91·4	93·1	90·0

The experimental and theoretical values are plotted in Fig. 2, and it can be seen that the rate of fall in recovery with sampling time is approximately the same. The fact that the experimental results are invariably about 4 per cent. below the theoretical may be regarded as a measure of the mechanical efficiency of the bubbler system.

Dimensions of solvent container and delivery tube—Solvent containers having cross-sectional areas of 0·5 and 1·0 sq. cm showed only slight differences in absorption efficiency; a nominal size of 1-cm internal diameter (equivalent to an area of 0·8 sq. cm) was adopted for development of the bubbler design. Variations of ±25 per cent. from the nominal 2 ml of solvent were found to have no significant effect.

Various types of delivery tube were tested (see Fig. 3); type C—the thick-walled capillary tube—gave a slightly better recovery than type A, and type B was definitely inferior to

both A and C. Variations in jet diameter between 0·5 and 1·5 mm produced no difference in absorption efficiency. Thick-walled capillary tube of 1-mm internal diameter (nominal) was adopted; this had the additional advantage over the other types tested of being more robust. Subsequently it was found necessary to specify tubing of 1·0 to 1·5-mm internal diameter, as tubing of less than 1·0 mm was found to interfere with the operation of the 1 litre per minute critical orifice (see later).

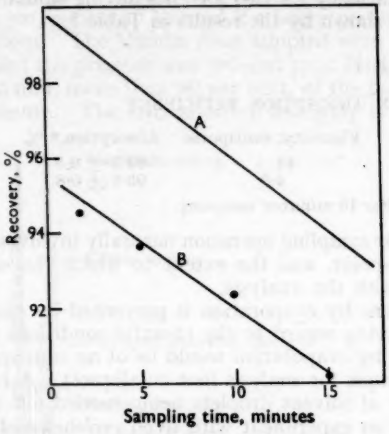

Fig. 2. Effect of sampling time on absorption efficiency: curve A, theoretical results; curve B, experimental results

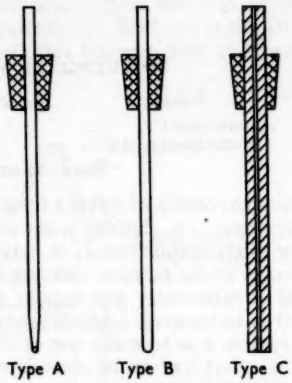

Fig. 3. Experimental inlet tubes for miniature bubbler

Effect of temperature—The implication of the theoretical treatment given above is that absorption efficiency will fall with increasing temperature owing to the increase in the saturated vapour concentration of the agent. The magnitude of this effect is shown by the following theoretical recovery values for Sarin vapour at a constant sampling time of 5 minutes—

Temperature, °C	15	20	25	30
Theoretical recovery, % ..	98·9	98·5	98·0	97·2

In practice, however, the mechanical efficiency of the bubbler system might be expected to increase with increasing temperature owing to the fall in viscosity of the solvent. The viscosity of *cyclo*hexanol is about 70 centipoise at 20° C and falls to about half this value

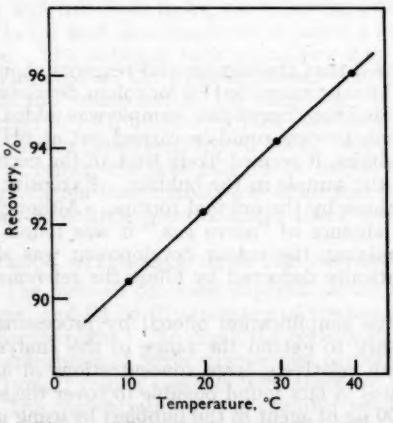

Fig. 4. Effect of solvent temperature on absorption efficiency

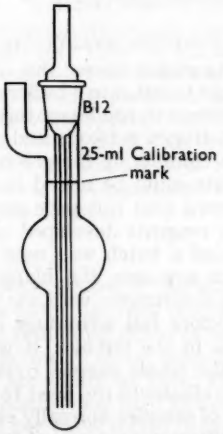

Fig. 5. Miniature bubbler

with a 10° C rise in temperature. The results of one series of tests, in which the solvent was maintained at various temperatures, are shown in Fig. 4, a small increase in efficiency with rise in temperature being indicated. This trial was not representative of sampling in various climatic conditions, as the air temperature remained constant before entry into the bubbler. It seems likely, however, that the two effects resulting from increased temperature would be to some extent compensating.

The influence of viscosity on absorption efficiency for two solvents having similar molecular weights but widely different viscosities is shown by the results in Table I.

TABLE I

INFLUENCE OF VISCOSITY ON ABSORPTION EFFICIENCY

Solvent	Molecular weight	Viscosity, centipoise	Absorption,* %
*cyclo*Hexanol	100	49	93·2 ± 0·5
4-Methylpentanol-2 ..	102	4·0	96·1 ± 0·8

* Mean of four tests each after 10 minutes' sampling.

The final choice of solvent for any particular sampling operation naturally involves other considerations, *e.g.*, boiling-point and freezing-point, and the extent to which the solvent itself or likely impurities in it may interfere with the analysis.

Design of the bubbler—Serious loss of solvent by evaporation is prevented by choosing a liquid of sufficiently low vapour pressure, having regard to the climatic conditions under which it is to be used. Small losses of solvent by evaporation would be of no consequence, as the system was to make use of the entire sample for analysis (not an aliquot). A further possibility that had to be considered was that of solvent droplets being carried out of the bubbler by the air stream. It was shown by an experiment with dyed *cyclo*hexanol that, with a tube of 1 cm diameter and 6 cm long, losses due to spray could be entirely avoided by inclusion of a small bulb at the top of the tube.

One of the most time-consuming steps in the original procedure was the removal of liquid from the bubbler and measurement of a suitable aliquot. As explained below, it was found possible with the new system to make the bulb at the top of the solvent container of a size such that the vessel could serve the dual purpose of bubbler and 25-ml calibrated flask, which permits the sample to be analysed *in situ*.

The bubbler is shown in Fig. 5. In the original design, the capillary tube was held in position by a small rubber bung; this had the attraction of cheapness and would no doubt be quite satisfactory for many purposes. For "nerve-gas" sampling, however, it was found that in transit to and from the field solvent occasionally came into contact with the rubber and extracted from it materials that interfered with the analysis. An all-glass design was therefore adopted; in this the capillary tube fitted into the solvent container with a standard B12 ground-glass joint.

THE MODIFIED ANALYTICAL PROCEDURE—

As stated above, the original method required that the sample and reagents should be brought together in a buffer at pH 5·5 and the pH finally raised to 11·0 for colour development. Experience in the laboratory showed that, provided the "nerve-gas" sample was added after the hydrogen peroxide and *o*-dianisidine, the whole process could be carried out at pH 11·0. When considering the procedure for the new bubbler, it seemed likely that all the necessary reagents could be mixed in bulk and added to the sample in the bubbler. Extensive tests confirmed that this gave results identical with those by the original routine. Although the mixed reagents developed colour slowly in the absence of "nerve gas," it was found that, provided a batch was used within 1 hour of mixing, the colour development was slight, and, in any case, this blank colour was automatically deducted by filling the reference cell in the colorimeter with the mixed reagent.

Before full advantage could be taken of the simplification offered by processing the sample in the bubbler, it was obviously necessary to extend the range of the analysis so that the whole sample could be used, even with relatively large concentrations of agent, *i.e.*, to eliminate the need to take aliquots. It was in fact found possible to cover the entire range of samples normally encountered (0 to 1000 μg of agent in the bubbler) by using in the absorptiometer a range of optical cells of widths from 0·25 to 3·0 cm.

CONTROL OF FLOW RATE—

The critical orifices were originally hand-made by constricting one end of a glass tube and checking the flow rate against an accurate flowmeter of the Rotameter type. Even when those with flow rates between 0·8 and 1·2 litres per minute were accepted, a considerable proportion had to be rejected. Much more consistent results were obtained by using discs cut from glass tubing of guaranteed internal diameter (Veridia tubing obtained from Chance Brothers Ltd.). Ideally the orifice should be in a very thin plate, but in practice thicker plates (or discs) may be used if a larger pressure difference is maintained to give critical flow conditions. The Veridia discs adopted were $\frac{1}{16}$ inch thick, and critical flow was maintained provided the pressure was reduced to at least half an atmosphere. With an orifice diameter of 0·35 mm, more than 90 per cent. of the discs gave flow rates between 0·95 and 1·00 litres per minute. The critical-orifice assembly is shown in Fig. 6.

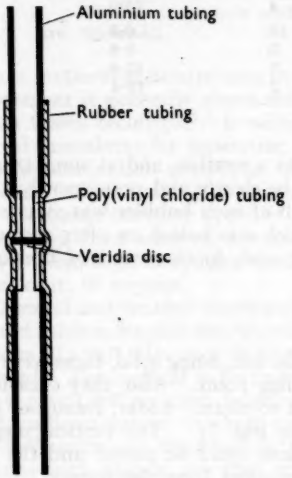

Fig. 6. Critical-orifice assembly

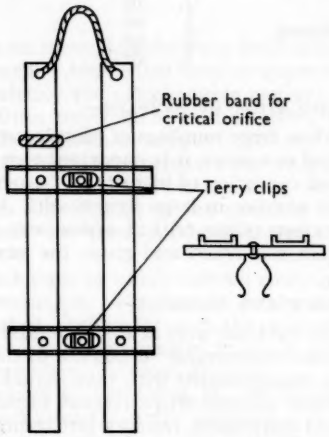

Fig. 7. Metal holder for bubbler

As mentioned above, one object in the development of the new system was to arrange that checking the performance of the equipment should, as far as possible, be completed before leaving the laboratory. To make sure that each critical orifice was correctly assembled, clean and free from leaks, the following procedure was devised. A pump, similar to those used in the field, was employed to power a manifold fitted with 10 connections and a pressure gauge. The bubblers each containing 2 ml of solvent were fitted with their critical orifices and mounted on the bubbler frames (see below), which were suspended in order along the bench and connected to the manifold. A roving lead from a Rotameter flowmeter was attached to each bubbler in turn and the flow rate entered against the appropriate bubbler number in a permanent-record book. As any given bubbler was always used with the same critical orifice, the flow rate to be expected could be obtained from the previous record; any deviation from normal was noted and its cause investigated. It was found that a team of two with little practice could check about 300 bubblers in an hour.

PRECISION ATTAINABLE WITH THE PROPOSED SYSTEM—

The over-all precision depends (*a*) on the consistency of performance of the sampling system, and (*b*) on the accuracy of the analytical method. The latter has been discussed in a separate paper.[1] To check the performance, a large number of trials with different nominal concentrations of Sarin vapour was carried out in the 100 cubic metre static chamber. A few typical results together with those by the original method are shown in Table II.

The concentration in the chamber would of course be appreciably less than the nominal concentration, which was simply the weight of agent dispersed per cubic metre of chamber

capacity, owing to loss of vapour on the walls. During the preliminary trials, percentage absorption in a single bubbler was assessed by operating two bubblers in series and analysing the samples in both. When sufficient results had been collected to assign a "slip correction" for any given sampling time, bubblers were operated singly and the necessary corrections applied.

TABLE II

PRECISION ATTAINABLE WITH THE PROPOSED SYSTEM

System	Number of samples	Nominal concentration of Sarin, mg per cubic metre	Sampling time, minutes	Mean concentration of Sarin found, mg per cubic metre	Mean deviation, %
Original	3	25	4	14·8	9
	3	25	4	14·5	5
	3	25	4	16·5	3
	3	25	4	15·0	10
Proposed	10	7·8	10	6·5	1·1
	10	12·2	7	9·8	2·5
	10	20·9	5	17·6	1·7
	10	24·0	2	19·4	1·5

IDENTIFICATION OF SAMPLES—

When large numbers of samples are being handled as a routine, and at some stages by unskilled operators, it is important that all items should be clearly and permanently marked to avoid confusion of identity. To achieve this, the bulb of each bubbler was marked with a serial number in large figures with Araldite paint, which was baked on after application. The corresponding critical orifice was similarly marked, and, in the event of breakage, a new item was fitted and given the same number.

ASSEMBLY AND CARRIAGE—

The bubblers were at first mounted on plywood boards, but, being solid, these were found to produce undesirable turbulence in the air at the sampling point. Also, they occasionally became contaminated with toxic liquid and were difficult to clean. Later, frames of ½-inch aluminium channel-strips riveted together were used (see Fig. 7). The vertical members provided convenient recesses into which the critical orifices could be placed and the Terry clips permitted bubblers to be quickly attached to or removed from the frames.

The projections on the horizontal strips were of uniform length to allow the complete assembly to be a loose fit in the slots of plywood boxes for carriage to and from the field.

CONCLUSIONS

A novel system for sampling and determination of toxic vapour in the field has been developed. Its particular application to the "nerve gases" is considered, although it has since been successfully used for chlorine, phosgene, cyanogen chloride and mustard gas.

The sampling equipment could without doubt be used for any vapour dispersed in the atmosphere, but one of the most attractive features of the system is the simplicity achieved by carrying out the analysis without removing the sample from the bubbler. How far this could be realised in any other application depends on the method of analysis of the particular agent.

The work described was carried out under the direction of Mr. L. T. D. Williams.

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The Determination of the Oxygen Content of Beryllium Metal by Activation

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The oxygen content of beryllium metal was found by irradiating samples with 14·5-MeV neutrons and measuring the 7·4-second nitrogen-16 activity produced in the reaction $^{16}O\ (n,p)\ ^{16}N$. Oxygen contents in the range 0·01 to 2·0 per cent. have been measured by this method. As little as 0·001 per cent. of oxygen can be detected, provided that the intensity of short-lived reaction products from other impurities is sufficiently low.

The identification and determination of other impurities in the metal are discussed.

Several methods of determining oxygen in beryllium are in use, but the lower limit of accurate measurement is generally about 0·1 per cent. of oxygen. Above this level of impurity, the vacuum fusion technique[1,2] is widely used and is probably the most reliable method. Two chemical procedures for separating beryllium metal from beryllium oxide have been utilised for the determination of oxygen. Wallace[3] dissolved beryllium in copper sulphate solution, beryllium oxide remaining as an insoluble residue, but other analysts have found this method to be unreliable for some types of sample. Corbett's procedure[4] for determining oxygen in titanium has been adapted successfully to beryllium. Distillation of the metal in chlorine leaves a residue of beryllium oxide. The lower limit for these methods is again about 0·1 per cent. of oxygen.

Osmond and Smales[5] developed an activation technique in which thermal neutrons were used and lithium fluoride was mixed with the beryllium sample. Fluorine-18, produced by the reactions $^{6}Li\ (n,t)\ ^{4}He$ and $^{16}O\ (t,n)\ ^{18}F$, was separated chemically and the activity was measured. The method is limited to fine powders and its lower limit of accurate measurement is about 0·2 per cent. because of the oxygen impurity in lithium fluoride.

A radioactivation method for determining oxygen in beryllium by irradiation with 14·5-MeV neutrons is described in this paper. The massive metal can be analysed, and surface reactions with oxygen are thus minimised. The high sensitivity of the method makes it particularly useful for determining traces of oxygen in pure beryllium.

Description of the Method

The method used was to irradiate the beryllium sample in a 14·5-MeV neutron flux and then to count the residual activity with a Geiger counter. The 7·4-second β^- activity[6] from nitrogen-16 produced in the reaction $^{16}O\ (n,p)\ ^{16}N$ was then compared with the activity from a similar-sized sample containing a known added amount of oxygen.

Neutrons of 14·5 MeV were obtained by bombarding a tritiated zirconium target with 500-KeV deuterons. The total neutron output from the target was maintained at approximately 10^{10} neutrons per second. Short irradiations of the order of 1 to 10 seconds were used, the sample being in a standard position close to the neutron source. These irradiations were made with the help of a fast mechanical shutter in the deuteron beam; the shutter was operated by magnetic solenoids to ensure a sharp rise and fall at the beginning and end of an irradiation. The neutron output was monitored by means of a "long counter,"[7] the pulses being fed via a ratemeter into a fast pen recorder. The neutron output and the time of irradiation were measured on the recorder chart.

After irradiation, samples were transferred to a standard position near a 2-inch diameter end-window Geiger counter, the output from which was fed alternately into two scalers every 5 seconds. Scaler readings were recorded in the alternate periods during which the instruments were not receiving pulses. The transfer of the sample to the counter and the recording of the counter output were done manually, as the number of samples to be examined did not justify the installation of elaborate automatic handling and counting equipment.

For routine analysis, it would be more convenient to count the particles from the samples with a counter having a short dead time, such as a proportional or scintillation counter.

The output from such a counter could be recorded directly on a fast pen recorder via a logarithmic ratemeter.

As an alternative to beta-particle detection, the high-energy gamma rays from nitrogen-16 could be counted in a large sodium iodide crystal. However, the size of beryllium sample required to increase the counting rate in order to compensate for the low detection efficiency would generally be too large.

Calibration of standard beryllium

A cross-section of 49 $\pm$ 25 millibarns has been found by Paul and Clarke[8] for the reaction ^{16}O (n,p) ^{16}N. A more precise value would permit the oxygen content of a beryllium sample to be determined absolutely, *i.e.*, without reference to samples of known oxygen content. However, to do this, an absolute neutron-flux measurement and accurate absorption and scattering corrections for the nitrogen-16 β^- particles in the beryllium sample would be necessary. These could only be obtained with precision for a thin sample of beryllium, the weight of which would be insufficient to allow the determination of extremely small amounts of oxygen. It was therefore necessary to standardise the method by irradiating samples of known mixtures of beryllium and beryllium oxide.

Such mixtures were prepared by thorough mixing in an atmosphere of argon. After they had been compacted and sintered in a vacuum, the samples were machined to a standard size (1 cm in diameter and 0·5 cm in length). This overcame the need for corrections to take into account the variation in neutron dose, counter geometry and self-absorption of beta-particles, which would be necessary if samples of different sizes were used. The samples examined contained 0, 0·32, 0·65, 0·96, 1·28 and 1·60 per cent. w/w of added oxygen. Three samples at each concentration were prepared.

Each sample was irradiated for a few seconds and then counted as described. A typical decay curve is shown in Fig. 1. The 7·4-second component from nitrogen-16 and a longer-lived component from impurities in the beryllium are evident. The 7·4-second activity of each sample was normalised to a standard neutron flux and time of irradiation and was corrected for the small variations in the weight of each sample. The results are shown in

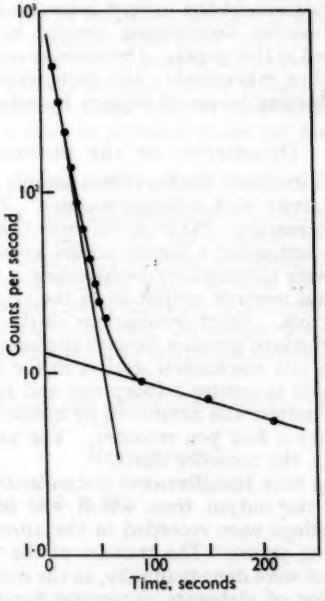

Fig. 1. Beta-particle decay curve for a sample of beryllium containing 0·64 per cent. of oxygen after irradiation with 14·5-MeV neutrons for 2·0 seconds

TABLE I

ANALYSIS OF KNOWN MIXTURES OF BERYLLIUM AND BERYLLIUM OXIDE

Amount of oxygen added (A), %	Normalised activity, counts per minute	Activity due to added oxygen (B), counts per minute	Ratio B/A, counts per minute	Difference of B/A from mean counts per minute
0	20,800	—	—	—
	20,800	—	—	—
	22,900	—	—	—
0·32	43,400	21,900	68,400	+ 9900
	41,200	19,700	61,600	+ 3100
	39,200	17,700	55,300	− 5200
0·65	64,600	43,100	66,300	+ 7200
	63,100	41,600	63,100	+ 4600
	66,100	44,600	68,600	+10,100
0·96	74,500	53,000	55,200	− 3300
	79,400	57,900	60,300	+ 1800
	74,600	53,100	55,300	− 3200
1·28	85,500	64,000	50,100	− 8400
	100,000	78,500	61,300	+ 2800
	85,500	64,000	50,000	− 8500
1·60	101,500	80,000	50,100	− 8400
	99,200	77,700	48,700	− 9800
	98,000	76,500	47,900	−10,600
		Mean ..	57,500	σ .. 7135

$$\text{Standard deviation} = \frac{7135 \times 100}{57{,}500} = 12{\cdot}4 \text{ per cent.}$$

Table I. The scatter of the three values measured at each concentration is due partly to counting statistics and timing errors and partly to the difficulty in preparing samples of known and uniform beryllium oxide content.

From the results in Table I, the oxygen content of the beryllium powder used to prepare the standards was found to be 0·38 ± 0·05 per cent. This agrees with the mean value of 0·47 ± 0·13 per cent. found from a series of determinations by the vacuum fusion technique on the same powder. A sample of this beryllium was used as a standard in subsequent determinations.

The error for the standard, ±12 per cent., is absolute, whereas the relative error in comparing two samples, say, before and after a purification procedure, would include only the counting and timing errors. The counting errors cannot be improved beyond the point at which the counting rates are seriously reduced by the dead time of the counter used. Provided, therefore, that this maximum counting rate is obtained for each sample by adjusting the neutron flux according to the oxygen content, the relative error will remain the same, *i.e.*, about 5 per cent. With the maximum neutron flux available, the lowest oxygen content detectable was approximately 0·001 per cent. No great improvement in the sensitivity of the method can be obtained by increasing the size of the sample. This is because of self-absorption by the beryllium of the nitrogen-16 beta particles (half-thickness ~ 1 g per sq. cm). If the oxygen distribution in the sample is not homogeneous, the accuracy of the method will be decreased. In the extreme, if all the oxygen were concentrated in a small volume on one of the faces of the cylinder, the oxygen determination would be in error by a factor of 2.

Seven samples from along the length of a zone-refined bar of beryllium were analysed, the calibrated sample being used for comparison; the results were as follows—

Sample No.	1	2	3	4	5	6	7
Oxygen content, % ..	0·012	0·008	0·008	0·008	0·008	0·010	0·016
Direction of zone melting ..				⟶			

The smooth variation in oxygen content is an indication of the reproducibility of the method at low levels of oxygen impurity.

ERRORS CAUSED BY REACTIONS WITH IMPURITIES

The presence of other impurities that give rise to reaction products with half-lives of the order of 5 to 15 seconds would, of course, invalidate the oxygen content found by the proposed method.

A search of the Table of isotopes revealed five such elements that could occur in beryllium metal, *viz.*, sulphur, silicon, aluminium, magnesium and fluorine. To ensure that the oxygen content of a sample, as measured, is not in error by more than 10 per cent., these elements must not be present to an extent greater than, respectively, 1, 20, 10, 10 and 0·1 times the oxygen impurity. These figures have been calculated from published nuclear data and some unpublished cross-section measurements made by us. They are only approximate owing, mainly, to the inaccuracy of the published data on the cross-sections involved.

The amounts of some of these impurities present in a beryllium sample can be found by irradiating the sample for a relatively long time and examining the long-lived induced activity. A beryllium sample having a measured oxygen content of 0·01 per cent. was irradiated for 30 minutes and the decay curve was analysed into five components with half-lives of 7·4 seconds, ~2·5 minutes, ~10 minutes, ~2·5 hours and ~15 hours. An analysis of this decay curve is shown in Table II, from which it can be seen that all the components can be accounted for by amounts of impurities that agree with the results of a chemical analysis. The amounts of aluminium, silicon and mangesium impurities are all much less than the maximum permissible levels previously mentioned.

Table II

Analysis of the decay curve for a beryllium sample

Impurity present	Reaction	Half-life of reaction product	Calculated amount of impurity present, %	Amount of impurity found by chemical analysis, %
Aluminium ..	$^{27}Al\ (n,\alpha)\ ^{24}Na$	15 hours	~0·04	0·04
	$^{27}Al\ (n,p)\ ^{27}Mg$	10 minutes		
Silicon	$^{28}Si\ (n,p)\ ^{28}Al$	2·3 minutes	~0·02	0·03
Magnesium ..	$^{25}Mg\ (n,p)\ ^{25}Na$	1 minute	<0·004	0·003
Iron	$^{56}Fe\ (n,p)\ ^{56}Mn$	2·6 hours	~0·05	0·05

Fluorine and sulphur could not be detected from the decay curve. As a chemical analysis set upper limits of 0·0005 per cent. for these two elements, they could not cause any appreciable errors in the oxygen determination. This beryllium sample was therefore sufficiently free from all the elements likely to cause serious errors.

Conclusions

The proposed procedure is the only method yet developed that is capable of the precise determination of amounts of oxygen less than 0·1 per cent. in beryllium. It is possible to measure as little as 0·005 per cent. of oxygen with an error of ± 20 per cent. The chief disadvantage is that the samples to be analysed and the standards for comparison must be the same shape and must therefore be machined.

The method is applicable to other materials, provided that the half-lives of the fast-neutron reaction products of the main constituents are sufficiently different from that of nitrogen-16.

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The Determination of Copper in Certain High-alloy Steels

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A modified method for the determination of copper in certain high-alloy steels has been developed. Separation is based on the extraction of copper dithizonate with carbon tetrachloride, copper being finally determined absorptiometrically as its blue bis*cyclo*hexanone oxalyldihydrazone complex. The range of copper concentration covered by the method is about 2 to 10 per cent., but this can be extended in either direction by adjusting the sample weight or the aliquot of solution used in the extraction.

The procedure described in this paper was devised in order to provide an accurate means of determining copper in certain high-alloy steels. Methods in which bis*cyclo*hexanone oxalyldihydrazone is used for the determination of copper in ferrous materials have been described by Haywood and Sutcliffe[1] and Wetlesen.[2] We have successfully used Haywood and Sutcliffe's procedure to determine copper in a range of low-alloy and mild steels, but, when the method was applied to the analysis of permanent-magnet alloys and stainless steels, a significant fading of the blue colour was observed, especially with the magnet alloys. Previous workers[1,2] have recorded that iron produces a background colour under the experimental conditions, which is compensated for by the blank solution, and that sexavalent chromium does not cause any significant interference. Wetlesen[2] noted that large amounts of cobalt and nickel cause a negative interference, and consequently it was simple for us to confirm that these two metals were the cause of the fading in our experiments. We were able to show that cobalt was responsible for a more serious error than nickel and that, for the magnet alloys, the average order of the fading was about 5 to 7 per cent. of the theoretical optical density in 30 minutes. Optical density - time graphs were plotted and it was shown that the theoretical optical density was obtained if the graphs were projected to zero time. Consequently, the error caused by fading can be reduced by measuring the optical density of the solution as soon as possible after colour development is complete. This expedient is only a partial solution to the problem and cannot conveniently be resorted to when analyses are being carried out in batches or when results of the highest accuracy are required. In order to overcome the problem of fading, attempts were made to introduce a suitable separation procedure. The use of a dithizone extraction stage has permitted us to modify the method successfully.

Experimental

Solvent-extraction procedures were reviewed, and it appeared that copper could be selectively extracted, as its dithizone complex,[3] from a solution of the steel in dilute acid. Attempts to determine copper directly by measuring the optical density of the extract were unsuccessful, as dithizone absorbs strongly in the same waveband as the copper complex and the mixed-colour method is not sufficiently accurate. It is possible to remove excess of dithizone by means of an aqueous ammoniacal solution, but this results in the loss of some copper by partial keto - enol transformation in the complex.[4] Consequently, it was impossible to use a direct measurement on the carbon tetrachloride extract.

A modification of the method was therefore required, and, provided that iron, cobalt and nickel were not co-extracted to any significant extent with the copper dithizonate, it appeared that the bis*cyclo*hexanone oxalyldihydrazone complex could be used. Tests were carried out to determine the behaviour of these three metals. Solutions of 0·1-g samples of a synthetic alloy of iron, cobalt and nickel (1 + 1 + 1) were prepared and extracted in exactly the same way as for the removal of copper from a solution of a steel. The resulting organic extract was analysed for copper and nickel by polarography and for iron by absorptiometry, and it was shown that the concentrations of all three metals were in the range 0·01 to 0·1 p.p.m.

It was therefore clear that bis*cyclo*hexanone oxalyldihydrazone could be used to determine copper after separation as its dithizonate. It was simple to destroy the organic ligand with an oxidising acid mixture before the blue colour of the copper complex was developed.

METHOD

REAGENTS—

Nitric acid, diluted (1 + 3).

Sulphuric acid, sp.gr. 1·84.

Carbon tetrachloride.

Dithizone solution—Prepare a 0·05 per cent. solution of dithizone in carbon tetrachloride.

Oxidising acid mixture—Mix 1 volume of sulphuric acid, sp.gr. 1·84, 1 volume of nitric acid, sp.gr. 1·42, and 2 volumes of perchloric acid, sp.gr. 1·70.

Citric acid solution—Prepare a 25 per cent. w/v solution of citric acid in water.

Ammonia solution, sp.gr. 0·880.

α-Naphtholphthalein indicator solution—Prepare a 0·1 per cent. solution of the indicator in 50 per cent. aqueous ethanol.

*Bis*cyclo*hexanone oxalyldihydrazone solution*—Prepare a 0·5 per cent. solution of the reagent in 50 per cent. aqueous ethanol.

Standard copper solution—Prepare a solution containing 1 mg of copper per 10 ml by dissolving spectrographically pure copper in diluted nitric acid (1 + 1). The final solution used was approximately 0·1 *N* with respect to nitric acid.

PROCEDURE—

Dissolve 0·1 g of alloy in 10 ml of diluted nitric acid (1 + 3) in a 125-ml beaker. (For samples of stainless steel, use 50 ml of diluted sulphuric acid (1 + 4), and oxidise residual carbides with about 5 ml of nitric acid, sp.gr. 1·42.) Cool the solution, and carefully add 10 ml of sulphuric acid, sp.gr. 1·84, except for samples of stainless steel, when no further sulphuric acid need be added. Cover the beaker with a watch-glass, heat until fumes of sulphur trioxide are evolved, cool to room temperature, and dilute to 500 ml in a calibrated flask. Transfer 10 ml of the resulting solution to a 100-ml separating funnel, and dilute to 30 ml. (If the sample contains less than about 2 per cent. of copper, take a larger aliquot, and dilute accordingly to the correct acidity, 0·1 to 0·5 *N*.[3])

Extract successively with 30-ml and 5-ml portions of 0·05 per cent. dithizone solution and, finally, with 10 ml of carbon tetrachloride, which removes the residual dithizone and permits the analyst to ascertain that removal of copper has been quantitative. Combine the extracts in a 125-ml conical beaker, and add 4 ml of oxidising acid mixture. Heat gently to remove carbon tetrachloride and then strongly to destroy any remaining organic material. Ensure that the solution is evaporated until fumes are evolved, but do not heat excessively; large variations in the concentration of ammonium salts present cause small errors at low concentrations of copper. Cool to room temperature, add 5 ml each of distilled water and 25 per cent. citric acid solution, adjust the pH to between 8·0 and 9·5 with ammonia solution, sp.gr. 0·880 (see Note), and transfer the solution to a 100-ml calibrated flask. Place the flask in a thermostatically controlled water bath at 20° C, and add 5 ml of 0·5 per cent. bis*cyclo*hexanone oxalyldihydrazone solution. (A temperature of 20° C was arbitrarily chosen for the dilution of all solutions to a standard volume in order to avoid the difficulties encountered at lower temperatures, *e.g.*, below 15° C formation of the copper complex is sometimes delayed.) Shake the flask, allow the colour to develop for 5 minutes, and dilute to the mark with distilled water at 20° C.

Shake well, and measure the optical density of the solution in a 1-cm cell at 595 mμ with a spectrophotometer that has been set to zero against water. Read the copper content of the alloy from a calibration graph plotted from a range of copper solutions prepared by dilution of the standard copper solution. The most useful range of copper concentration in the final solution is approximately 40 to 200 μg per 100 ml.

NOTE—Use wide-range indicator papers in strips of about ⅛-inch to adjust the pH. Carry out a check by placing 1 drop of test solution and 1 drop of α-naphtholphthalein indicator solution about 0·5 cm apart on a filter-paper. As the drops merge, a distinct blue colour is observed when the pH of the test solution is in the correct range. Any indeterminate colours or colours produced when the spots approach each other but do not merge must be ignored. This procedure was devised to overcome the effects of ammonia evolved from the buffered solutions, which should be stoppered if set aside for any length of time.

TABLE I

DETERMINATION OF COPPER IN STANDARD ALLOYS

Alloy	Element present	Amount of element present, %	Certified copper content, %	Amount of copper found, %
Stainless steel—				
B.C.S. No. 235	Si	0·82	0·98	0·96, 0·95, 0·99, 0·98, 0·98
	Mn	0·46		
	Ni	8·73		
	Cr	19·01		
	Ti	0·62		
	W	0·68		
Permanent-magnet alloys—				
B.C.S. No. 266	Si	0·22	3·33	3·30, 3·32, 3·35, 3·30, 3·35, 3·33, 3·25
	Mn	0·27		
	Ni	13·3		
	Co	23·4		
	Al	7·95		
	Nb	1·21		
B.C.S. No. 233	Si	0·57	5·09	5·05, 5·07, 5·12, 5·09, 5·12, 5·03
	Mn	0·235		
	Ni	11·22		
	Co	23·72		
	Al	6·98		
	Ti	0·79		

RESULTS

To illustrate the precision of the method, recoveries of copper from British Chemical Standard alloys are shown in Table I. A Unicam SP500 spectrophotometer was used to measure the optical densities of the test solutions.

The range of copper concentration covered by the proposed procedure is approximately 2 to 10 per cent., but can be conveniently extended in either direction by varying the sample weight or the aliquot of sample solution and altering the concentrations of the reagents accordingly. The optical density of the final solution can then be controlled so that maximum accuracy is obtained from the scale readings.

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A Rapid Method for the Determination of Total Sulphur in Soils and Plants

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A statistical study of the use of turbidimetric measurements of sulphate as barium sulphate is described. Procedures are given for the determination of total sulphur in soils and plants, extremely pure magnesium nitrate being used to oxidise the organic matter. The techniques are eminently suitable for large batches of samples and should have general application in biological and geochemical analysis.

MOST agricultural chemists agree that there is an urgent need for a rapid method for determining total sulphur in the materials they handle. This was made apparent when Beauchamp[1]

pointed out that the waters of Lake Victoria were deficient in sulphate and that the soils of Uganda might also be deficient. Sulphur deficiencies had been suspected from anomalous results of fertiliser experiments and were later confirmed by pot experiments.

Little[2] has recently reviewed methods for determining sulphur in soils and has described a *ferrum reductum* procedure for total sulphur. Destruction of organic matter by oxidation offered prospects of greater simplicity, but the use of perchloric acid was precluded, as, in the hands of unqualified African staff, the risks of explosions were considered to be too high. Several fusion methods involving sodium peroxide were tried, but all were given up in despair (compare MacIntire[3]). Magnesium nitrate[4] offered good prospects as an oxidant on the micro scale, but early results were disappointing, as blank values were high. Specpure magnesium rod as a source of magnesium nitrate finally led to safe rapid methods, which are almost completely free from reagent blank values.

The proposed method is based on that of Chesnin and Yien[5] for available sulphate in soils. A statistical approach to the study of the variables involved has resulted in several improvements to their turbidimetric procedure and its adaptation to the determination of total sulphur.

Experimental

Effect of operational variables—

Crystal size of barium chloride—A series of standards containing 0, 100, 200, 300, 400 and 500 μg of sulphur was prepared in duplicate in the presence of 5 ml each of 50 per cent. acetic acid and 25 per cent. nitric acid; six grades of barium chloride crystals were used. Optical-density measurements were made after the standards had been set aside for 1 hour. The results were statistically analysed, and Table I shows the values of the correlation coefficient, or multiple correlation coefficient when appropriate, and the coefficient of variation for each series. Crystals of size 1 mm to 52-mesh B.S.S. were selected as being the most suitable.

Table I

Effect of barium chloride crystal size on relationship between optical density and sulphur level

Crystal size	Correlation coefficient for— 1-cm cell	Correlation coefficient for— 4-cm cell	Coefficient of variation for— 1-cm cell, %	Coefficient of variation for— 4-cm cell, %
1 mm to 36-mesh B.S.S. ..	$R_{123} = 0{\cdot}9977$	$r = 0{\cdot}9997$	5·3	1·9
36-mesh B.S.S. to 0·5 mm ..	$R_{12} = 0{\cdot}9961$	$r = 0{\cdot}9994$	7·1	3·5
0·5 mm to 52-mesh B.S.S. ..	$R_{12} = 0{\cdot}9989$	$r = 0{\cdot}9976$	2·9	5·3
52-mesh B.S.S. to 70-mesh I.M.M.	$r = 0{\cdot}9932$	$r = 0{\cdot}9934$	10·4	9·1
0·5 mm to 70-mesh I.M.M. ..	$r = 0{\cdot}9878$	$r = 0{\cdot}9922$	9·8	6·8
1 mm to 52-mesh B.S.S. ..	$R_{123} = 0{\cdot}9986$	$r = 0{\cdot}9992$	5·2	2·9

Batch variation of barium chloride crystals—Different batches of barium chloride crystals gave rise to slightly different constants in the regression equation relating optical density to level of sulphur. It is therefore preferable to prepare a standard series for each batch of crystals.

Type and concentration of acid—A suitably buffered medium is necessary to ensure a favourable rate of dissolution of barium chloride, as this influences the optical density and reproducibility of the barium sulphate cloud. Hydrochloric acid - acetic acid mixtures were found to be less satisfactory than nitric acid - acetic acid mixtures. The addition of orthophosphoric acid, although primarily intended to decolorise iron in soil extracts, resulted in an optical-density increase of about 9 per cent. and a linear relationship between optical density and level of sulphur. The effects of variation in the amounts of acetic and nitric acids on the determination of 250 μg of sulphur in the presence of 1 ml of orthophosphoric acid after a standing time of 1½ hours are shown in Table IIA and values of the least significant difference in Table IIB.

Table IIa

Effect of different amounts of acetic and nitric acids on the determination of 250 μg of sulphur

Each result is the mean of three determinations and is referred to a standard series prepared in presence of 5 ml each of 50 per cent. acetic acid and 25 per cent. nitric acid and 1 ml of orthophosphoric acid

Amount of 25 per cent. nitric acid present, ml	Amount of sulphur found— in absence of acetic acid, μg	in presence of 5 ml of 50 per cent. acetic acid, μg	in presence of 10 ml of 50 per cent. acetic acid, μg	Average amount of sulphur found, μg
0	185·7†	166·3†	158·7†	170·2
2·5	262·0†	252·3†	239·3†	251·2
5	241·0	243·3	246·3	243·6
7·5	211·7	232·0	240·7‡	228·1
10	177·3‡	205·0‡	227·7‡	203·3
Average	215·5	219·8	222·5	

† Turbidity developed rapidly.
‡ Dissolution of barium chloride was incomplete and barium orthophosphate was precipitated.

Table IIb

Values of the least significant difference

Effect	Least significant difference at— 5 per cent. level of significance	1 per cent. level of significance	0·1 per cent. level of significance
Acetic acid	4·3	5·8	7·7
Nitric acid	5·6	7·5	9·9
Interaction	9·6	13·0	17·2

The results show that, in the presence of either 5 or 10 ml of 50 per cent. acetic acid, minor fluctuations in the concentration of 25 per cent. nitric acid about the 5-ml level will have no serious effect on the determination. As the solubility of barium chloride is lower in the presence of 10 ml of 50 per cent. acetic acid, the 5-ml level was chosen as being the more suitable.

Time of standing—The effect of time of standing after dilution to volume on the optical density of the barium sulphate cloud was investigated by using a standard series prepared in duplicate covering the range 0 to 1000 μg of sulphur in 125-μg stages. The results, summarised in the form of regression equations, are shown in Table III.

Table III

Effect of time of standing on optical density of barium sulphate cloud

Optical-density measurements were made with a Spekker absorptiometer. The relationship between optical density and sulphur level is given by the expression $y = a + bx$, where y is absorptiometer reading × 1000 and x is the amount of sulphur present in micrograms

Time of standing, hours	Sulphur level between 0 and 500 μg† a	b	σ_b	Sulphur level between 0 and 1000 μg‡ a	b	σ_b
1	7·0	2·190	±0·020	3·1	0·5464	±0·0028
1·5	12·0	2·151	±0·017	6·3	0·5301	±0·0018
2	16·1	2·116	±0·014	9·4	0·5181	±0·0029
2·5	26·8	2·061	±0·020	12·7	0·5100	±0·0042
3	27·0	2·006	±0·021	11·5	0·5004	±0·0072
3·5	26·6	1·965	±0·024	11·1	0·4894	±0·0037
4	29·0	1·910	±0·031	7·3	0·4980	±0·0045
5·5	35·2	1·880	±0·031	0·9	0·4900	±0·0063
7	27·4	1·841	±0·045	1·1	0·4904	±0·0065

† Optical densities measured in 4-cm cell. ‡ Optical densities measured in 1-cm cell.

The results show that the optical density decreases when the solutions are set aside and that the standard error of the regression coefficient passes through a minimum during the period 1½ to 2 hours; the regression coefficients for these two periods are not materially different, and a standing time of 1½ hours was therefore adopted.

Optical filter—The optical density of the barium sulphate cloud is best measured with use of a blue filter, as the values are numerically higher and are less subject to random variations. The presence of a yellow colour in some soil extracts precludes the use of a filter transmitting at 425 or 465 mμ. It was found, however, that the absorption of the coloured soil extracts was high at these two wavelengths, passed through a minimum at 490 mμ and rose to a secondary maximum at 545 mμ. In view of this behaviour, optical density was measured at 490 mμ so that the same standard series would be applicable to both soils and plants.

EFFECT OF FOREIGN IONS—

The effects of considerable excesses of some foreign ions likely to occur in agricultural samples were investigated. Their effects on the determination of 250 μg of sulphur are shown in Table IV, from which it can be seen that, in practice, only large amounts of calcium are likely to cause interference. Interference from silica is not likely, as both soils and plant materials receive an acid treatment that will largely result in its removal, and, in this connection, nitric acid is to be preferred to hydrochloric acid.

TABLE IV

EFFECT OF FOREIGN IONS ON SULPHUR DETERMINATION

Added ion	Form of added ion	Amount of ion added, milli-equivalents	Effect on determination of 250 μg of sulphur, μg
Mg^{2+}	MgO	6·4	0·0
		8·0	− 3·3
		9·6	− 7·0*
NO_3^-	$Mg(NO_3)_2$	6·4	− 1·7
		8·0	− 6·7
		9·6	− 1·0
Ca^{2+}	$CaCO_3$	2	+ 1·7
		4	+ 3·7
		8	− 2·7
		16	−10·3**
Na^+	$NaOOC{\cdot}CH_3$	5	− 3·5
		10	+ 1·5
K^+	$KOOC{\cdot}CH_3$	5	− 1·0
		10	− 5·0
Fe^{3+}	$Fe(OOC{\cdot}CH_3)_3$	5	− 6·3
		10	−23·3***
Al^{3+}	$Al(OOC{\cdot}CH_3)_3$	5	+ 5·4
		10	+ 3·0
Mn^{2+}	$Mn(OOC{\cdot}CH_3)_2$	5	+ 6·7
		10	−13·0***
Cl^-	NaCl	5	+ 1·3
		10	−12·4***
		20	−18·4***
$(H_2PO_4)^-$	NaH_2PO_4	5	− 1·0
		10	− 3·0
$(SiO_3)^{2-}$	Na_2SiO_3	1	+ 2·0
		2	+ 2·6
		5	+20·3***

* Statistically significant at the 5 per cent. level of significance.
** Statistically significant at the 1 per cent. level of significance.
*** Statistically significant at the 0·1 per cent. level of significance.

METHOD

REAGENTS—

Magnesium nitrate solution—Dissolve 25 g (5 rods) of Specpure magnesium in 400 ml of analytical-reagent grade nitric acid in a 1500-ml flask. If any magnesium remains undissolved

when the reaction has finished, add a further 50 ml of nitric acid. Add 100 ml of water to dissolve the crystals of magnesium nitrate, cool, and dilute to 500 ml. This solution should be clear and colourless.

Nitric acid, fuming—Analytical-reagent grade.

Nitric acid, 31·2 per cent. v/v—Prepare from analytical-reagent grade nitric acid.

Nitric acid, 25 per cent. v/v—Prepare from analytical-reagent grade nitric acid.

Acetic acid, 50 per cent. v/v—Prepare from analytical-reagent grade acetic acid.

Orthophosphoric acid—Analytical-reagent grade.

Gum acacia solution—Dissolve 5 g of gum acacia in 1 litre of hot water. Filter the hot solution through a Whatman No. 42 filter-paper. Always check this solution for clarity before use, and re-filter if necessary.

Standard sulphur solution, 50 μg per ml—Dissolve 0·3844 g of magnesium sulphate, $MgSO_4.7H_2O$, in water, and dilute the solution to 1 litre.

Barium chloride—Grind analytical-reagent grade barium chloride crystals to pass through a 1-mm sieve, and remove smaller particles with a 52-mesh sieve.

PREPARATION OF STANDARDS—

Place 0, 2, 4, 6, 8, 10, 12, 16 and 20 ml of standard sulphur solution in a series to 50-ml calibrated flasks. Place 5 ml of 50 per cent. acetic acid, 5 ml of 25 per cent. nitric acid and 1 ml of orthophosphoric acid in each flask, dilute to about 48 ml, and shake well.

From a graduated dispensing tube, add 1 g of barium chloride crystals to each flask without disturbing the contents. Set each flask aside for 10 minutes, and then invert it twice. Repeat the inversion after a further 5 minutes, and, 5 minutes later, invert each flask ten times, add 1 ml of gum acacia solution, dilute to volume, and invert twice. Set each flask aside for 1½ hours, invert ten times, and measure the optical densities in both 4-cm and 1-cm cells with a Spekker absorptiometer at 490 mμ. The relationship between optical density and level of sulphur is linear. The standard graph is applicable to subsequent determinations made with the same batch of barium chloride crystals.

PROCEDURE FOR SOILS—

Weigh 1 g of 0·5-mm soil into a lipless 50-ml tall beaker. Add 2 ml of magnesium nitrate solution, and evaporate excess of moisture and acid in an electric oven at 70° C. Heat overnight at 300° C in a stainless-steel oven. Cool, add 5 ml of 31·2 per cent. nitric acid, cover the beaker with a well fitting watch-glass, and digest on a water bath for 2½ hours. Avoid loss of nitric acid.

Transfer the cooled digest quantitatively to a 50-ml calibrated flask, dilute to volume, shake well, and filter through a dry fluted Whatman No. 42 filter-paper. Place a 40-ml aliquot of the filtrate in a 50-ml calibrated flask, add 5 ml of 50 per cent. acetic acid and 1 ml of orthophosphoric acid. Shake well, and develop turbidity in the way described for the standards.

PROCEDURE FOR PLANT MATERIAL—

Weigh 0·2 g of finely ground plant material into a 50-ml tall beaker. Add 2 ml of fuming nitric acid, cover the beaker with a watch-glass, and set aside overnight. Wash the watch-glass, add the washings to the beaker, and evaporate to dryness on a steam-bath. Add 2 ml of magnesium nitrate solution, evaporate excess of moisture, and place in a cold muffle furnace. Heat overnight at 450° C.

In the cold beaker place 5 ml of 25 per cent. nitric acid, and, when dissolution is complete, transfer to a 50-ml calibrated flask, and dilute to volume. Filter through a dry Whatman No. 42 filter-paper. Place a 20-ml aliquot of the filtrate in a 50-ml calibrated flask. Add 5 ml of 50 per cent. acetic acid, 3 ml of 25 per cent. nitric acid and 1 ml of orthophosphoric acid. Dilute to about 48 ml, mix, and develop turbidity as previously described.

RESULTS

Results for the reproducibility and recovery of added sulphur for nine soils and five plant materials are shown in Tables V and VI. Recovery of sulphur added as sulphate and methionine is quantitative within the limits of experimental error. The average coefficient of variation is 3·1 per cent. for soils and 1·7 per cent. for plants.

TABLE V

RECOVERY OF SULPHUR ADDED TO 1-g SAMPLES OF SOILS

Each result is the mean of four determinations

Sample	Amount of sulphur added— as $MgSO_4$, μg	Amount of sulphur added— as methionine, μg	Amount of sulphur found, μg	Amount of added sulphur recovered, μg	Coefficient of variation, %
A	—	—	11·3 ± 0·9	—	15·7
	100	—	114·0 ± 1·7	102·7 ± 1·9	3·0
	—	100	112·5 ± 2·3	102·2 ± 2·4	4·0
B	—	—	30·3 ± 0·9	—	5·7
	100	—	131·3 ± 2·8	101·0 ± 3·0	4·3
	—	100	127·8 ± 3·1	97·5 ± 3·2	4·9
C	—	—	89·3 ± 0·9	—	1·9
	100	—	190·0 ± 2·7†	100·7 ± 2·8	2·4
	—	100	184·3 ± 2·1	95·0 ± 2·3	2·3
D	—	—	124·3 ± 1·5	—	2·4
	100	—	232·0 ± 3·0	107·7 ± 3·4	2·6
	—	100	226·8 ± 2·0	102·5 ± 2·5	1·8
E	—	—	232·0 ± 6·2	—	5·3
	100	—	326·8 ± 4·5	94·8 ± 7·7	2·8
	—	100	327·0 ± 5·9	95·0 ± 8·6	3·6
F	—	—	248·5 ± 4·5	—	3·6
	100	—	355·8 ± 4·0	107·3 ± 6·0	2·3
	—	100	341·0 ± 3·8	92·5 ± 5·9	2·2
G	—	—	325·0 ± 3·4	—	2·1
	100	—	419·5 ± 2·0	94·5 ± 3·9	0·9
	—	100	418·8 ± 3·4	93·8 ± 4·8	1·6
H	—	—	380·0 ± 5·7	—	3·0
	100	—	496·3 ± 7·6	116·3 ± 9·5	3·1
	—	100	474·5 ± 4·7	94·5 ± 7·4	2·0
I	—	—	473·0 ± 2·7	—	1·1
	100	—	577·3 ± 11·5	104·3 ± 11·8	4·0
	—	100	575·5 ± 5·7	102·5 ± 6·3	2·0

Average recovery of sulphur added as $MgSO_4$ = 103·3 ± 6·5 μg
Average recovery of sulphur added as methionine = 97·2 ± 5·3 μg
Average coefficient of variation = 3·1 per cent.

† Mean of three determinations.

TABLE VI

RECOVERY OF SULPHUR ADDED TO 0·2-g SAMPLES OF PLANT MATERIAL

Each result is the mean of four determinations

Sample	Amount of sulphur added— as $MgSO_4$, μg	Amount of sulphur added— as methionine, μg	Amount of sulphur found, μg	Amount of added sulphur recovered, μg	Coefficient of variation, %
Millet A	—	—	231·5 ± 2·9	—	2·5
	200	—	435·3 ± 3·1	203·8 ± 4·2	1·4
	—	200	435·3 ± 1·8	203·8 ± 3·4	0·8
Millet B	—	—	940·0 ± 9·6	—	2·1
	200	—	1133·3 ± 9·5	193·3 ± 13·5	1·7
	—	200	1139·3 ± 5·1	199·3 ± 10·9	0·9
Coffee (Arabica)	—	—	360·0 ± 4·6	—	2·5
	200	—	565·5 ± 6·1	205·5 ± 7·6	2·2
	—	200	554·5 ± 2·9	194·5 ± 5·4	1·0
Coffee (Robusta)	—	—	493·8 ± 4·9	—	2·0
	200	—	699·0 ± 4·5	205·2 ± 6·6	1·3
	—	200	698·5 ± 3·9	204·7 ± 6·3	1·1
Maize	—	—	382·8 ± 2·3	—	1·2
	200	—	577·8 ± 7·2	195·0 ± 7·5	2·5
	—	200	585·0 ± 2·6	202·2 ± 3·5	0·9

Average recovery of sulphur added as $MgSO_4$ = 200·6 ± 8·5 μg
Average recovery of sulphur added as methionine = 200·9 ± 6·5 μg
Average coefficient of variation = 1·7 per cent.

Discussion of the method

A reagent blank should be run with each series of determinations, which should preferably be performed in duplicate, as occasional determinations may be in error by more than 10 per cent. Violent shaking can cause errors of up to 10 per cent., but slow inversion leads to reproducible results. The pre-treatment of plant material with fuming nitric acid is necessary to prevent losses of sulphur by volatilisation as ammonium sulphate. Some soils give pale straw-coloured extracts, possibly due to the presence of titanium. An appropriate correction for colour may be applied by determining the optical density of the solution after barium sulphate has been removed by filtration through a dry Whatman No. 42 filter-paper. The correction is small with a filter transmitting at 490 mμ.

In the development of the procedure for soils, dissolution in hydrochloric acid and then precipitation of sesquioxides with ammonia led to variable results, partly owing to the effect of chloride ion and partly to absorption of sulphate by the precipitate. Results were unsatisfactory when the ignited residue was leached with either hydrochloric or nitric acid, and filtration was slow. Digestion with nitric acid was satisfactory both from the viewpoints of consistent results and ease of manipulation.

An ashing temperature of 450° C is suitable for both plants and organic soils. Lower temperatures, *i.e.*, down to 300° C, can be used for mineral soils and graminaceous plants. The number of samples that can be dealt with in 1 day depends on the sizes of the oven and muffle furnace, but not more than thirty absorptiometric readings (at a rate of one per minute) should be made at one time.

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The Semi-micro Determination of Fluorine and Chlorine in Organic Compounds

Part III.* The Decomposition of Fluoro and Chlorofluoro Compounds by the Diphenyl-Sodium-Dimethoxyethane Complex

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Stable fluoro and chlorofluoro compounds can be quantitatively decomposed by the diphenyl-sodium-dimethoxyethane complex. The sodium salts are extracted with water, and the chloride and fluoride are determined after passage through a cation-exchange resin.

For some time it has been evident that the quantitative decomposition of some organic fluorine-containing compounds, such as benzotrifluoride and its derivatives and chlorofluoro-*cyclo*hexanes, cannot always be performed with success by the accepted technique of fusion with sodium or potassium at 500° to 700° C. Results are often slightly low for compounds known to be pure. Sometimes this is caused by incomplete decomposition, and, at others, by slight leaks past the copper washers of the nickel bombs. When these bombs are used at the higher temperature, the nickel becomes soft and the bearing surfaces need to be faced after each determination in order to remove traces of copper, which sometimes produce channels through which leaks can occur. The use of even higher fusion temperatures[1,2]

* For details of Part II of this series, see reference list, p. 247.

accentuates, in our opinion, these mechanical difficulties, and, although fusion may more often be complete, failures from these other causes are more frequent.

An alternative method of decomposition, which has been extensively used for organic halogen compounds other than those containing fluorine, involves the use of ether - sodium - aromatic hydrocarbon complexes,[3,4,5,6] of which the most effective appear to be the naphthalene - sodium - dimethoxyethane and the diphenyl - sodium - dimethoxyethane complexes. Other aromatic compounds, such as anthracene and benzophenone,[4] and other ethers, such as dimethyl ether, diethyl ether, tetrahydrofuran, dioxan and ethylene glycol diethyl ether,[3,4,5] have been tried and rejected because the complex is less reactive, contains less sodium or does not keep for a sufficient length of time. Ma[7] has stated that the decomposition of fluorine-containing compounds by the diphenyl - sodium - dimethoxyethane complex has been reported,[8] but no details have been published.

Experimental

The proposed method is exactly the same in principle as the sodium - liquid ammonia method for the decomposition of organic fluorine compounds,[9,10] which has been rejected owing to the low solvent power of liquid ammonia. It therefore seemed worth investigating, despite earlier statements[3,5] that some fluorine-containing compounds do not react, and it was found (see Table I) that reproducible quantitative results were easily obtained for all the compounds tested, provided that they were soluble in di*iso*propyl ether. As the necessary solubility is only of the order of 0·1 per cent., this requirement has only been of importance in the analysis of highly fluorinated polymers, which are so insoluble that they cannot be analysed in this way and must be decomposed by the fusion method. When polytetrafluoroethylene was heated under reflux in di*iso*propyl ether, the decomposition when a large excess of diphenyl - sodium - dimethoxyethane reagent was added was less than 10 per cent.

The procedure for decomposition was almost the same as that used by Liggett,[5] except that di*iso*propyl ether was used as the solvent for the organic compound. Toluene, benzene, and benzene - diethyl ether mixtures were tried, but were rejected because emulsions were formed during the extraction of the sodium salts with water. Diethyl ether was rejected because the heat evolved during decomposition of the excess of reagent caused losses in the ether spray when the pressure in the separating funnel was released. The sodium salts were extracted with water instead of nitric acid and the determination was completed by a modification of the procedure described by Banks, Cuthbertson and Musgrave,[11] an aliquot of the extract being passed through a cation-exchange resin, the total acid determined by alkalimetry and the chloride by the mercury oxycyanide method. The fluoride was then found by difference.

Table I

Amounts of halogen found by the proposed method

Compound	Formula	Amount of fluorine— present, %	Amount of fluorine— found, %	Amount of chlorine— present, %	Amount of chlorine— found, %	Amount of bromine— present, %	Amount of bromine— found, %
Benzotrifluoride	$C_7H_5F_3$	39·0	39·0	—	—	—	—
Fluorobenzene	C_6H_5F	19·7	19·9	—	—	—	—
3-Nitro-4-aminobenzotrifluoride	$C_7H_5O_2N_2F_3$	27·6	27·5	—	—	—	—
2-Chloro-5-trifluoromethylacetoacetanilide	$C_{11}H_9O_2NClF_3$	21·1	20·9	13·1	13·0	—	—
3-Amino-4-ethanesulphonylbenzotrifluoride	$C_9H_8O_4NF_3S$	20·1	20·1	—	—	—	—
Dihydrodecafluoro*cyclo*hexane	$C_6F_{10}H_2$	72·0	71·6	—	—	—	—
Trihydrononafluoro*cyclo*hexane	$C_6F_9H_3$	69·5	69·6	—	—	—	—
Monochloroundecafluoro*cyclo*hexane	$C_6F_{11}Cl$	66·0	65·7	11·2	11·3	—	—
Dichlorodecafluoro*cyclo*hexane	$C_6F_{10}Cl_2$	57·1	57·1	21·4	21·2	—	—
Trichlorononafluoro*cyclo*hexane	$C_6F_9Cl_3$	48·9	48·6	30·4	30·6	—	—
Tetrachloro-octafluoro*cyclo*hexane	$C_6F_8Cl_4$	41·5	41·6	38·8	28·4	—	—
Pentachloroheptafluoro*cyclo*hexane	$C_6F_7Cl_5$	34·8	35·0	46·4	46·3	—	—
Bistriethylphosphine-*p*-trifluoromethylphenylbromopalladium	$C_{19}H_{34}P_2PdBrF_3$	10·0	9·9	—	—	14·1	13·7

It is most important to use a concentrated solution of the complex (1 to 1·5 *M* in sodium) for the decomposition and to store it, as described by Liggett, in small sealed bottles in a refrigerator. We have found that, after several portions have been taken from a bottle and it has been kept for a short time at room temperature, it becomes much less efficient. When fresh and concentrated, it remains green when added to the di*iso*propyl ether solution and heat is developed immediately, but, after it has been kept, addition to the di*iso*propyl ether gives a brown colour (which changes to green) and the heat of reaction develops much more slowly, sometimes about 2 minutes being required. When this happens, it is better to start again with fresh reagent, as decomposition may be incomplete.

The conversion of sodium salts to acids by passing the solution through a column of Amberlite IR-120(H) has been altered from that previously described,[11] the amount of resin being reduced to the minimum necessary to convert each aliquot and fresh resin being used for each determination. This is because the aqueous extract of sodium salts inevitably contains some organic solvents, which contaminate the resin so that it cannot be completely regenerated. Results were good after the resin had been regenerated once, but subsequent regenerations were unsuccessful.

Method

PROCEDURE—

Add an amount of the organic compound equivalent to about 8 mg of total halogen to 20 ml of di*iso*propyl ether in a 100-ml separating funnel fitted with a well ground tap. Weigh the compound in a small gelatin capsule, the lid of which can be separated from the base as the two are dropped into the ether. When the compound has dissolved, add 10 ml of a 1 to 1·5 *M* solution of the diphenyl - sodium - dimethoxyethane complex, prepared as described by Liggett,[5] and set aside for 10 minutes with occasional swirling. The reaction mixture should become warm immediately and remain green. Shake with about 20 ml of water, and run the lower aqueous layer into a 100-ml calibrated flask. Wash the stem of the separating funnel carefully, and repeat the extraction three times. Dilute to the mark, and, by pipette, place a 25-ml aliquot of the solution on about 5 g of Amberlite IR-120(H) in a glass column 8 mm in diameter shaped as previously described.[11] Elute with 70 ml of boiled distilled water over a period of 10 minutes, heat the effluent to boiling, and cool immediately. Titrate the whole effluent with 0·02 *N* sodium hydroxide (use methyl red screened with either bromocresol green or methylene blue as indicator).

If more than one halogen is present, proceed as described by Banks, Cuthbertson and Musgrave.[11]

Carry out a blank determination on the complex solution every second day.

We thank Professor G. E. Coates for discussions on the reactions of alkali metal - hydrocarbon complexes, and the Imperial Smelting Corporation Ltd., Avonmouth, for pure samples of some of the compounds analysed.

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NOTE—Reference 11 is to Part II of this series.

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A Universal Buffer Solution for Use in Ultra-violet Spectrophotometry

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Replacement of the barbitone component of Britton and Robinson's universal buffer mixture by trishydroxymethylaminomethane allows this buffer to be used for pH control in ultra-violet spectrophotometry. A pH - composition Table for the use of this buffer at 25° and 37° C is given for the pH range 2 to 12.

Ionic strength, dilution and buffer capacity results for these solutions are also provided.

An essential requirement of any buffering medium for use in ultra-violet absorptiometry is that it should not adversely affect the useful range of transparency of the pure solvent. This condition precludes the use of such highly light-absorbent buffer components as phenylacetic and diethylbarbituric acids, the spectra of which exhibit strong absorption bands ($\epsilon > 100$ litres per mole per cm) in this region.[1,2] The buffer solutions of Prideaux and Ward[3] and of Britton and Robinson[4] therefore require modification before they can be used in general ultra-violet spectrophotometry.

The ultra-violet spectra of aqueous solutions of trishydroxymethylaminomethane (2-amino-2-hydroxymethylpropane-1:3-diol) possess high uniform transmittance, and this base was found to be a suitable substitute for the barbitone of Britton and Robinson's buffer mixture. Trishydroxymethylaminomethane is a stable solid, which is readily soluble in water and crystallises from aqueous ethanol in the form of long needles; these contain no solvent of crystallisation and melt between 169·5° and 171·5° C. The buffer action of the base was first studied by Gomori,[5] who obtained a value for pK_b of 5·76 at 23° C. Bates and Pinching[6] have reported a value for pK_b of 5·92 at 25° C. Bates and Bower[7] have used trishydroxymethylaminomethane in the preparation of a standard one-component buffer solution covering the pH range 7 to 9, the solution forming one of several intended for use in alkaline pH control.

A suitably modified formulation of Britton and Robinson's buffer mixture for use over the pH range 2 to 12 is described in this paper. A 10-mm path length of this buffer solution can be used at wavelengths down to 230 mμ in conjunction with commercial spectrophotometers working at their normal sensitivities. The Van Slyke buffer capacities, β,[8] Bates dilution values, $\Delta pH_{\frac{1}{2}}$,[9,10] and light-absorption characteristics of these solutions are shown in Figs. 1 and 2.

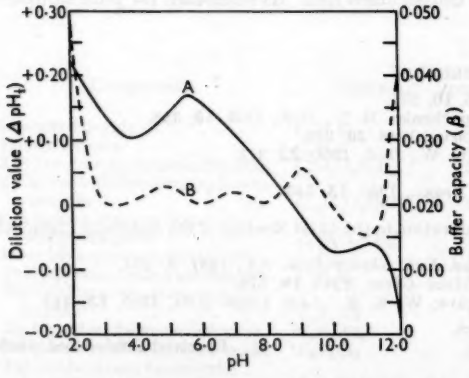

Fig. 1. Dependence on pH of dilution value, $\Delta pH_{\frac{1}{2}}$, and buffer capacity, β, of the universal buffer solution: curve A, values of $\Delta pH_{\frac{1}{2}}$; curve B, values of β

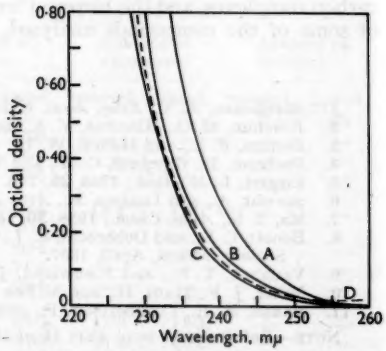

Fig. 2. Absorption spectra relative to air of the universal buffer solution at various pH values measured in cuvettes of 10-mm path length: curve A, pH 2·97; curve B, pH 5·70; curve C, pH 8·25; curve D, pH 10·75

The variation in approximate ionic strength of the buffer solution throughout the pH range was calculated from Bates and Pinching's value for pK_b at 25° C and from the pK_a data summarised by Vogel.[11]

Experimental

Preparation of reagents—

With the exception of the sample of trishydroxymethylaminomethane, the materials used were of analytical-reagent grade. All solutions were prepared with distilled water that had been stored in absence of carbon dioxide. Sodium tetraborate was recrystallised from water, dried in air and stored over a solution saturated with respect to both sucrose and sodium chloride. Carbonate-free solutions of 0·1 and 0·4 *N* sodium hydroxide were prepared by spinning (1 + 1) suspensions of water and pellets in a centrifuge and making the necessary dilutions from the clear supernatant liquid. These solutions were standardised by titration against 0·4 *N* hydrochloric acid, which was itself standardised against solid sodium tetraborate.

Trishydroxymethylaminomethane was recrystallised from 20 per cent. aqueous ethanol and dried to constant weight at 80° to 90° C *in vacuo* over phosphorus pentoxide. The purity of the sample was shown by acid titration to be not less than 99·7 per cent.

Citric acid and potassium tetroxalate were used without further purification. Potassium chloride, potassium dihydrogen orthophosphate and "anhydrous" disodium hydrogen orthophosphate were dried in an oven, the first two at 115° to 120° C and the last at 180° to 185° C. Potassium hydrogen phthalate was recrystallised from water and dried at 115° to 120° C.

For measurements at 25° C, solutions and standards were prepared at laboratory temperature and equilibrated in a thermostatically controlled water bath for 30 minutes before use. Determinations of pH at 37° C were carried out with solutions prepared and equilibrated at that temperature.

pH measurements—

All pH determinations were carried out on solutions contained in a water-jacketed cell controlled to within ±0·5° C at 25° and 37° C. The cell also contained a Pye universal glass electrode and the capillary liquid junction of a saturated potassium chloride - calomel - mercury reference electrode. Stirring and exclusion of carbon dioxide were effected by a stream of nitrogen. A Pye direct-reading pH meter was used, the output from which was fed through a precision 2000-ohm resistor placed in parallel with a Tinsley potentiometer, model 3184. This procedure ensured instrumental reproducibility of better than ±0·01 units of pH throughout the scale.

pH scale—

The pH scale chosen was that recommended by the United States National Bureau of Standards. The pH assembly was standardised by means of the solutions shown in Table I. Values at 37° C were obtained by interpolating results for these solutions at 35° and 40° C. These solutions are included in Tables of National Bureau of Standards buffer mixtures presented by Bates in chapter 4 of his monograph,[10] pp. 74 and 87. This chapter also contains a useful account of the original standardisation procedures with full references. Bates and Bower[7] have reported a liquid-junction error between pH 9 and 13 and have made allowance for this by assuming a linear relationship between error and pH in this region. This effect, which is caused by the presence in the liquid junction of a high concentration of extremely mobile hydroxyl ion, is additive to any residual non-linearity in the response of the lithia-glass membrane in alkaline solutions, an effect known to be small. A similar junction effect can, of course, be predicted for the region pH 2 to 4, but the magnitude of this was such that it was not detectable during the standardisation procedure.

Table I

Solutions used to standardise pH assembly

Solution	pH at 25° C	pH at 37° C
Potassium tetroxalate, 0·05 *M*	1·68	1·69
Potassium hydrogen phthalate, 0·05 *M*	4·01	4·02
Phosphate*	6·86	6·84
Sodium tetraborate, 0·01 *M*	9·18	9·06
Sodium hydroxide, 0·10 *N*	12·88	12·51

* A solution 0·025 *M* in both disodium hydrogen orthophosphate and potassium dihydrogen orthophosphate.

TABLE II

CHARACTERISTICS OF THE FINAL BUFFER SOLUTIONS

Amount of 0·4 *N* hydrochloric acid added, ml	Amount of 0·4 *N* sodium hydroxide added, ml	pH of final solution at 25° C	pH of final solution at 37° C	Ionic strength, mole per litre
34·8	0·0	2·00	2·02	0·10
30·4		2·20	2·21	—
26·6		2·40	2·40	—
23·8		2·60	2·60	—
21·6		2·80	2·79	—
19·6		3·00	2·98	0·09
17·6		3·20	3·18	—
15·8		3·40	3·38	—
14·0		3·60	3·58	—
12·0		3·80	3·78	—
10·0		4·00	3·98	0·09
7·8		4·20	4·18	—
5·6		4·40	4·38	—
3·6		4·60	4·58	—
1·6		4·80	4·78	—
	0·4	5·00	4·99	0·11
	2·8	5·20	5·19	—
	5·0	5·40	5·39	—
	7·4	5·60	5·58	—
	9·4	5·80	5·78	—
	11·4	6·00	5·98	0·14
	13·4	6·20	6·18	—
	15·6	6·40	6·37	—
	17·8	6·60	6·56	—
	20·2	6·80	6·76	—
	22·4	7·00	6·96	0·21
	24·0	7·20	7·15	—
	26·6	7·40	7·34	—
	28·6	7·60	7·53	—
	30·8	7·80	7·72	—
	33·2	8·00	7·91	0·25
	35·6	8·20	8·10	—
	37·6	8·40	8·29	—
0·0	39·8	8·60	8·47	—
	43·4	8·80	8·66	—
	46·2	9·00	8·83	0·27
	49·0	9·20	9·03	—
	52·0	9·40	9·22	—
	54·6	9·60	9·42	—
	56·8	9·80	9·61	—
	59·0	10·00	9·81	0·29
	60·4	10·20	10·00	—
	61·6	10·40	10·20	—
	62·8	10·60	10·38	—
	64·0	10·80	10·58	—
	65·6	11·00	10·77	0·30
	67·0	11·20	10·97	—
	68·8	10·40	11·17	—
	71·0	11·60	11·36	—
	73·8	11·80	11·56	—
	77·2	12·00	11·76	0·33

OPTICAL MEASUREMENTS—

The optical densities of the buffer solutions, relative to air, were measured in 10-mm silica cuvettes over the range 225 to 400 mμ with a Unicam SP500 spectrophotometer.

COMPOSITION OF BUFFER SOLUTIONS—

A solution containing the following amounts of buffer components per litre was prepared—

Citric acid	21·01 g
Potassium dihydrogen orthophosphate	13·61 g
Sodium tetraborate	19·07 g
Trishydroxymethylaminomethane	12·11 g
Potassium chloride	7·46 g

A 50-ml portion of this solution was placed in a 200-ml calibrated flask, a specified amount of 0·4 *N* hydrochloric acid or 0·4 *N* sodium hydroxide was added and the solution was diluted to the mark with carbon dioxide-free distilled water. Table II shows the characteristics of the final buffer solutions prepared in this way.

I thank Dr. V. Petrow for his kind interest, and the Directors of The British Drug Houses Ltd. for permission to publish this paper.

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Notes

MODIFICATIONS TO A METHOD FOR THE DETERMINATION OF CHLORINATED HYDROCARBON PESTICIDE RESIDUES IN PLANT MATERIAL

A RECENT paper[1] from this laboratory describes the use of a modified semi-micro Stepanow procedure and then a mercuric oxycyanide titration of chloride for the determination of total organic chlorine in residues of chlorinated hydrocarbon pesticides. A colorimetric finish by the mercuric thiocyanate method[2,3,4] has been examined and found to be simpler and more rapid than the original procedure without loss of precision.

Tetrahydrofuran, the solvent previously specified for the modified Stepanow reaction can, for the colorimetric finish, be advantageously replaced by *n*-butylamine, which is more easily purified and strongly favours the formation of sodium naphthalene on which the reaction is based. The end-point of the reaction indicated by the intense colour of this compound is, with *n*-butylamine, usually apparent a few seconds after boiling begins, and a minimum boiling time of 1 minute after the appearance of the colour is recommended, as this was found to be sufficient to ensure complete dechlorination of such relatively stable compounds as DDT, benzene hexachloride, aldrin and dieldrin. It is necessary to remove most of the *n*-butylamine before the colorimetric stage of the determination, as it would otherwise cause a significant reduction in colour intensity.

Method

REAGENTS—

In addition to naphthalene, sodium and *iso*propyl alcohol, diluted (1 + 1), as specified previously,[1] the following reagents are needed—

Ammonium ferric sulphate reagent solution—Prepare an aqueous solution containing 12 g of analytical-reagent grade ammonium ferric sulphate and 40 ml of analytical-reagent grade nitric acid per 100 ml. Filter the solution if necessary.

Mercuric thiocyanate solution—Recrystallise mercuric thiocyanate from ethanol, and dissolve 0·4 g of the purified material in 100 ml of 95 per cent. ethanol.

n-Butylamine—To purify this reagent, heat a 50-ml portion under reflux with 2 g of naphthalene and 2 g of sodium cut into small pieces. When the liquid becomes dark red, transfer the flask to a water bath, and distil and collect the *n*-butylamine.

Nitric acid, diluted (1 + 2)—Prepare from analytical-reagent grade acid.

Diethyl ether—Analytical-reagent grade.

PROCEDURE—

Carry out the modified Stepanow procedure as previously described,[1] but use approximately 0·1 g of sodium, 0·25 g of naphthalene and 1·5 ml of *n*-butylamine in place of the tetrahydrofuran.

Allow the reaction to proceed until the boiling liquid becomes dark red, and then heat for a further 1 minute. Remove the excess of sodium as before by careful addition of 2 ml of *iso*propyl alcohol diluted (1 + 1), discontinue heating, detach the condenser, and transfer the flask to a steam-bath. (The neck of the flask should be steeply inclined to facilitate the escape of *n*-butylamine vapour.) When, after at least 30 minutes, most of the *n*-butylamine has been evaporated, remove and cool the flask, add a few millitres of water and then *p*-nitrophenol indicator and sufficient diluted nitric acid (1 + 2) to render the liquid acid. Transfer the liquid to a separating funnel, and shake with 20 ml of diethyl ether. Allow the layers to separate, run the aqueous layer into a 100-ml beaker, wash the ether layer successively with 10 and 5 ml of water, and add the washings to the beaker. Cover the beaker, introduce a narrow strip of polytetrafluoroethylene sheet or other device to prevent bumping, and heat to remove ether and reduce the volume to about 25 ml. Cool, transfer the liquid to a 50-ml stoppered and graduated cylinder, rinse the beaker with distilled water, and add the rinsings to the solution in the cylinder. Accurately note the volume, mix the solution, and if necessary, filter it through a sintered-glass filter or a filter-paper that has been previously washed free from chloride with distilled water and then dried. Check the optical density of the solution at 460 mμ, transfer not more than 20 ml to a 25-ml stoppered and graduated cylinder, accurately note the volume, and add 2 ml of ammonium ferric sulphate reagent solution and then 3 ml of mercuric thiocyanate solution. Make up the volume to 25 ml, and thoroughly mix. After 10 minutes, measure the optical density of the solution at 460 mμ in a cell of such a length that the reading is within the range covered by one of the calibration curves. Make an allowance, if necessary, for the optical density of the original solution, and deduct the optical-density blank value of the colorimetric reagent solutions made up to 25 ml with distilled water alone.

PREPARATION OF CALIBRATION CURVES—

For the lower range, prepare a standard potassium chloride solution containing 10 μg of chloride per ml. By pipette, place portions of this solution that contain up to 150 μg of chloride in 25-ml stoppered and graduated cylinders or calibrated flasks. Into each vessel in turn, place the colorimetric reagent solutions as described above, and make up the volumes to 25 ml. Mix the solutions, and measure the optical densities after 10 minutes in 2 or 4-cm cells at 460 mμ. Plot a graph of optical density, less that of distilled water *plus* the reagent solutions, against micrograms of chloride present. For the higher range, proceed in a similar manner, but use a standard potassium chloride solution containing 100 μg of chloride per ml and measure the optical densities in 1-cm cells for amounts of chloride up to 1000 μg. The relationship between optical density and chloride concentration is not linear, although it is approximately so when the amount of chloride present does not exceed 100 μg.

RESULTS

Organic chlorine determinations were carried out by the proposed method on known amounts of chlorinated hydrocarbon pesticides, which were introduced into the reaction flask by careful evaporation with an air current of portions of standard solutions in the presence of 2 drops of liquid paraffin. The results, corrected for a reagent blank value of 7 μg of chlorine, are shown in Table I.

TABLE I

RECOVERY OF CHLORINATED HYDROCARBON PESTICIDES BY THE PROPOSED METHOD

Pesticide	Amount of pesticide added, μg	Amount of pesticide recovered, μg
pp'-DDT	1000	983, 1027
	100	98, 98
γ-Hexachloro*cyclo*hexane	1000	1019, 1030
	100	96, 108
Aldrin	1000	1017, 986
	100	104, 102
Dieldrin	1000	1020, 1013
	100	102, 107

We thank the Government Chemist for permission to publish this Note.

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THE EQUIVALENT WEIGHTS OF WETTING AGENTS

In the examination of commercial wetting-agent compositions, an essential part of the procedure is the isolation of the active principle and the determination of its equivalent weight.[1] Since most such materials consist of monosodium salts of anionic wetting agents, the usual procedure is to convert a known weight of the material to sodium sulphate by ashing with sulphuric acid.[2] This procedure, as is well known, is subject to difficulties and is time-consuming. Anhydrous sodium sulphate tends to "creep" in platinum; it must be weighed rapidly because of its deliquescence. The ashing must be repeated with ammonium carbonate because of pyrosulphate formation.[2]

There is no general procedure for determining the equivalent weight of cationic wetting agents. Suitable possibilities are the determination of nitrogen by Kjeldahl's method or of halogen by Stepanow's method.[3]

This work shows that both classes of wetting agent can be made to yield an equivalent of titratable acid by passage through a cation-exchange resin.

Method

Procedure—

In a 2-cm diameter glass column fitted with a raised outlet tube (4 mm internal diameter) and tap,[4] prepare a bed of standard-grade Zeo-Karb 225, −16 to +50 mesh, on a small pad of glass-wool (a bed 14 to 15 cm deep is convenient). Convert the resin to the hydrogen form by passing 140 ml of 3 *N* hydrochloric acid through it at a rate of about 15 ml per minute. Wash with 500 ml of distilled water. Pass a further 50 ml of distilled water through the resin bed, and collect this separately. Add to it 2 drops of methyl red indicator solution and then 1 drop of 0·1 *N* sodium hydroxide. The indicator should turn clear yellow; if it does not, test further 50-ml washings in this way until it does. Warm the column by passing 100 ml of water at 60° C through it, and then place a clean 500-ml conical flask under the column. Place 50 ml of a warm aqueous solution containing a known amount of the wetting agent (0·5 g is normally suitable) on the column, and maintain the flow rate at 15 ml per minute. Wash with warm water until a total of 200 ml has been collected. Titrate with 0·1 *N* sodium hydroxide and methyl red as indicator. Use 50 ml of water in place of the sample solution to determine the blank value of the resin bed.

Discussion of results

In Table I, some results by the proposed method are compared with those by other methods. With the exception of cetyltrimethylammonium bromide, which was synthesised here, the wetting agents were commercial samples.

In all instances, the proposed method was more rapid than that with which it is compared. The only serious discrepancy is for Teepol 530. This is because Teepol of this type contains a proportion of potassium salts. For cetylpyridinium bromide, the difference from the theoretical value is presumably due to the use of a crude sample of cetyl bromide when the quaternary salt was prepared. The average difference (excluding Teepol 530) between results by the proposed method and the various alternatives is ±1·12 per cent.

TABLE I

COMPARISON OF RESULTS BY THE PROPOSED AND OTHER METHODS

Each result by the proposed method is the average of at least two determinations

Wetting agent	Theoretical equivalent weight	Equivalent weight found by— proposed method	Equivalent weight found by— alternative method	Alternative method used
Dioctyl sodium sulphosuccinate	444·6	442·9	445·0	Ashing with sulphuric acid
Diamyl sodium sulphosuccinate	360·4	360·0	359·2	
Dihexyl sodium sulphosuccinate	388·5	391·8	389·8	
4-*tert.*-Octylphenoxymethane sodium sulphonate	322·4	325·9	328·1	
Active material from Teepol 530*	—	337·3	294·8	
Active material from Teepol 410*	—	281·4	286·9	
4-*tert.*-Octylphenoxymethane ammonium sulphonate	317·4	308·2	313·5	Kjeldahl
Sodium tetradecan-2-sulphate	316·4	315·4	—	—
Sodium octadecan-2-sulphate	372·4	368·0	—	—
Cetyltrimethylammonium bromide	364·5	362·6	368·7	Stepanow
Cetylpyridinium bromide	384·4	409·0	402·8	Epton[5]

* The wetting agent contained a range of compounds of different chain length.

We thank J. A. Townsley and Mrs. J. D. Haynes for assistance with the experimental work, and the Directors of Ilford Limited for permission to publish this Note.

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RAMSDEN LABORATORY
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Book Reviews

THE U.S. FEDERAL FOOD, DRUG AND COSMETIC ACT (JUDICIAL AND ADMINISTRATIVE RECORD), 1953–1957. By VINCENT A. KLEINFELD and CHARLES WESLEY DUNN. Chicago, Illinois: Commerce Clearing House Inc. Pp. 1127. Price $25.00.

This handsome volume is the ninth research book in the Food Law Institute series and the fourth on this particular subject, which now covers the record from the passing of the Act in 1936 up to 1957.

The Analyst has already called attention to the earlier volumes in this series, and the latest work again provides invaluable information as a book of reference for the student of food and drug legislation. Naturally its content is of more practical value to our North American colleagues than to home readers, yet the book contains much of real interest. When one considers how rarely our own High Court is called upon to deal with this branch of legislation, it is remarkable to find that the record herein of leading cases from the U.S. courts covers 300 pages. Some of these are of more than academic interest, as witness the one that arose from the initiative of the Federal F and D. Commissioner in posting notices in every post office in the country, by way of information warning the public of the dangers of taking an alleged cancer cure. The Commissioner successfully defended an action brought against him by the proprietors for so doing. This long record of leading cases prompts the question whether our legislation must be less stringent than that of the U.S. our food and drug industry more law abiding or our enforcement less effective.

Food manufacturers in this country could hardly fail to be interested in the full record of official statements on F.D.A. general policy and interpretation that have been issued, for here many

of the problems dealt with are common to the industry the whole world over. This is supplemented by the record to date of Washington's new informal advisory service on the F.D.C. Act about subjects of trade interest, to be issued from time to time in the Federal Register. Further information of more general interest is given in relation to definitions and standards for foods, and to such topics as the official regulations dealing with habit-forming drugs (which can only be sold on prescription) and with tolerances for residues of pesticidal chemicals on raw agricultural commodities.

An earlier volume in this series reprinted the whole of the annual reports of the Food and Drug Administration from 1906 to 1949. The reports for 1950–1956, covering 175 pages, are included in this volume in order to bring the record up to date, and they are certainly of sufficient value and interest to justify their inclusion.

The value of this volume as a work of reference is greatly enhanced by the inclusion of a full and cumulative index, together with cumulative tables of statutes that cover the four volumes dealing with the administrative record. No doubt the authors are disappointed that the recent great advance of U.S. food legislation, *viz.*, the "added chemicals in food" amendment of the Act enacted in September last, could not be included for comment. This law now demands the pre-testing of all new chemical food additives by the manufacturer and their approval by the F.D.A. before use. But we shall hear more of this in due course.

It seems only a matter of justice to record that, although this volume is an expensive luxury for U.K. readers, the price still does not cover its cost, which has been subsidised by the Food Law Institute. One cannot but record appreciation of the industry and care that have gone into the production of this valuable work.

C. A. ADAMS

AUTOMATIC MEASUREMENT OF QUALITY IN PROCESS PLANTS. Proceedings of a Conference held at Swansea, September 23rd to 26th, 1957, by the Society of Instrument Technology. Pp. xii + 320. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1958. Price 50s.; $9.50.

These edited papers presented at the Society of Instrument Technology's 1957 Conference cover a wide range of topics, and, as they are presented by many authors, they necessarily vary in length, style and outlook. They range from the academic, J. Sheridan's paper on microwave spectroscopy, to the severely practical, C. W. Munday on a precision oxygen analyser; from a general survey of gas sampling by R. S. Medlock and H. Wilson, to a specialist paper on Wobbe index determinations by T. A. Lucas; from speculations on the use of X-ray fluorescent analysis by E. T. Hall, to details of operating a mass spectrometer as a process-stream analyser by D. S. Lees and D. E. Chalkley.

This wide range of papers indicates the progress made in the field of analytical instrumentation during the past few years. It is obvious that instruments are now very much a part of chemical processes and in one instance, that of distillation tests, have been accepted as suitable for use in a standard specification method.

The interest that the chemical industry is taking in automatic measurement is clearly shown by the fact that almost half the papers were presented by members of their staffs. It is from those papers that the difficulties of plant instrumentation can be gauged. The paper by J. P. Mole on infra-red gas analysers gives a good account of the types of problems encountered, problems often associated not with the instrument, but with such things as safety, choice of site, design of sample lines and maintenance procedure.

The instrument companies themselves are not unaware of the difficulties associated with plant instruments; the paper by R. S. Medlock and H. Wilson contains much information on gas sampling, and that by E. F. Daly on infra-red spectrometers clearly shows how aware the instrument designer is of plant conditions.

Finally the universities are also active in the field, and the papers by Dr. R. E. Richards on nuclear magnetic resonance, Dr. J. Sheridan on microwave spectroscopy and Professor W. Fishwick on uncommon techniques are evidence of their interest in instrumentation.

This volume then, although it cannot be considered a comprehensive textbook on instruments for quality control, is nevertheless a useful survey of the field. It is of particular interest when read in conjunction with the "Proceedings of the Congress on Modern Analytical Chemistry in Industry," held at St. Andrews in 1957. The two conferences taken together provide a comprehensive picture of the present state of development of analytical instruments.

The volume is well printed and bound, the only major printing error being the familiar

misprint of "calorimetric" for "colorimetric" beneath Fig. 3 on p. 12. The organisers of the conference and the publishers are to be complimented on having produced the book in a comparatively short space of time. It should be of interest and value to all chemists, physicists or engineers who deal with the development and use of analytical instruments for quality control, whether they be working in the laboratory or on the plant.

J. F. BROWN

AN INTRODUCTION TO PATHOLOGY. By G. PAYLING WRIGHT, D.M., F.R.C.P. Third Edition. Pp. xii + 660. London, New York and Toronto: Longmans, Green & Co. Ltd. 1958. Price 45s.

This book is essentially written for medical students to introduce a rational approach to clinical problems. In this, it has been a tremendous success, the two earlier editions being "best sellers." Although the book cannot be regarded as of direct concern to the analytical chemist, it is certain that those analysts dealing with forensic problems will find in it valuable information relevant to many of their tasks. Some knowledge of biological terminology by the reader is assumed, but there is much in the book that would be of interest to scientists in general. Professor Payling Wright is one of the few pathologists in the world capable of writing so erudite and interesting an account of the scientific principles upon which his subject depends.

C. H. GRAY

Publications Received

STANDARD METHODS FOR TESTING PETROLEUM AND ITS PRODUCTS. Eighteenth Edition. February, 1959. Pp. xxiv + 835. London: The Institute of Petroleum. Price 40s.

THE CHEMISTRY OF DRUGS. By NORMAN EVERS, Ph.D., F.R.I.C., and DENNIS CALDWELL, B.Sc., F.R.I.C. Third Edition. Pp. 415. London: Ernest Benn Ltd. 1959. Price 84s.

HANDBOOK OF CHEMISTRY AND PHYSICS. Edited by CHARLES D. HODGMAN, M.S., ROBERT C. WEAST, Ph.D., and SAMUEL M. SELBY, Ph.D. Fortieth Edition. Pp. xxiv + 3456. Cleveland, Ohio: Chemical Rubber Publishing Co. (Distributor in England: Blackwell Scientific Publications Ltd., Oxford.) 1958. Price $12.00; 100s.

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 38. Editor-in-Chief: JOHN C. SHEEHAN. Pp. viii + 120. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $4.00; 32s.

SOLUBILITIES: INORGANIC AND METAL-ORGANIC COMPOUNDS. A–IR. Volume I. By WILLIAM F. LINKE, Ph.D. Fourth Edition. Pp. iv + 1487. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Co. Inc. 1958. Price $25.00; 187s. 6d.

ELEMENTARY QUANTITATIVE ANALYSIS. By HAROLD F. WALTON. Pp. x + 342. London: Sir Isaac Pitman & Sons Ltd. 1958. Price 45s.

REPRINTS OF "THE INFRA-RED ANALYSIS OF SOLID SUBSTANCES"

By G. Duyckaerts

REPRINTS of the Review Paper, "The Infra-Red Analysis of Solid Substances," by G. Duyckaerts, published in this issue of *The Analyst* (pp. 201–214), will be available shortly from the Assistant Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1, at 2s. 6d. per copy, post free. A remittance for the correct amount, made out to The Society for Analytical Chemistry, MUST accompany the order; these reprints are not obtainable through Trade Agents.

"Recommended Methods for the Analysis of Trade Effluents": A Correction

IN the method for determining sulphide on p. 120 of the Society's publication "Recommended Methods for the Analysis of Trade Effluents," in the 5th and 6th lines under the heading "PROCEDURE," there is a sentence reading "If sulphite and thiosulphate are to be determined retain the precipitate." The last word should read "filtrate." This sentence does not occur in the version that appeared in *The Analyst*, 1957, **82**, 521 and as separate Reprint No. 10.

Erratum

MARCH (1959) ISSUE, p. 185, 6th line. *For* "water-soluble" *read* "water-insoluble."